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THE TACTILE HAIR OF THE WHITE RAT

S. B. VINCENT

From the Otological Laboratory of the Northwestern University Medical School

THIRTEEN FIGURES

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I. GENERAL DESCRIPTION

The principal tactile hairs of the white rat are found on the upper lip arranged in rows on either side of the nasal fossae. The longer and larger of these hairs are the most lateral ones of the second, third and fourth rows counting from below. Besides these there are a few scattered hairs on the lower lip, on the cheeks, above the eyes and on the fore limbs at the wrist joint. We are chiefly concerned with the vibrissae of the upper lip which in an active animal are in constant motion.

By the term 'hair' we usually mean the shaft which projects from the surface of the skin, but considered as a sense organ the important part is the follicle beneath the surface which encloses the base of the shaft. We may think of it as an invagination of the epidermis and see in it the usual skin layers somewhat modified under the different conditions of growth.

The follicle is a long oval in shape varying in length from 1 to 5 mm. and in width from 0.5 to 2 mm., and it is surrounded by two sheaths, a dermal and an epidermal. As we look at the follicle in a longitudinal section it appears like two pockets, one within

the other, which are separated by clear spaces or spaces crossed by connective tissue bridges—fine trabeculae (fig. 1). These cavities lie in a diverticulum of the fibrous, dermal, sheath and constitute the blood sinuses. The upper clear portion is known as the ring sinus (*e*), the lower as the venous or cavernous sinus (*j*).

In the lower part of the follicle may be seen an ingrowth of connective tissue which pushes the epithelial layers back and forms a central core at this place where the blood and lymph vessels can come into intimate contact with the growing portion of the hair. This is the papilla.

In the upper third of the follicle are the sebaceous glands which arise from the outer root sheath and whose ducts open upon the shaft of the hair. These glands lie above the ring sinus while below it or rather extending into it is an outgrowth from the root sheath named variously as the ringwulst, kissen, bourrelet annular, or pulvinus.

The follicle shows in a longitudinal section two distinct enlargements which are known as the superior and inferior swellings and the thickened portion of the root sheath above the sebaceous glands has been called the conical body (*b*).

Having looked at the salient features of this organ, we may now examine its structure more in detail. As has been said, the epidermal covering consists of two sheaths, an inner and an outer (fig. 2). The inner sheath corresponds to the stratum corneum of the epidermis and in good sections exhibits three layers of cells known respectively as the cuticle, Huxley's and Henle's layers. The latter, a continuation of the stratum lucidum, is frequently lacking. These cells differ somewhat from the usual epidermal cell layers, the two outer ones being nucleated while the cuticle is imbricated in such a way as to interlock with the plates of the cuticle of the hair shaft. For, as the tip of the invaginating hair column grows downward, an indentation is formed in it by the developing hair papilla "which is just sufficient to redirect the growth of the central hair column toward the cutaneous surface" which it reaches through a central canal formed by the degeneration of the central epidermal layer of the original hair column. According to this theory the cuticle of

the hair shaft is continuous with one of the layers of the inner root sheath and as its plates are turned in an opposite direction they interlock with the cuticle plates of the sheath. The cortical substance is a continuation of the stratum spinosum or middle Malpighian layer, while the cells of the medulla are formed by the proliferation of the cylindrical cells of the outer root sheath.

The outer root sheath is a continuation of the stratum germinativum and consists of three layers, a basement layer of cylindrical cells, the Malpighian layer of large prickle cells, and a granular layer of flattened cells which are often lacking. It is the cells of the second layer which increase so greatly in some parts of the follicle and are associated with the nerve endings. The dermal sheath also has three layers. Naming these from without inward they are a layer of longitudinal connective tissue fibers, a layer of circular fibers and the glassy layer. The last of these corresponds to the basement membrane of the derma. It is a clear, thick, highly refractive layer and the inner portion is said to be an exoplasmic product of the adjacent epithelium (Kölliker '02). The papilla has a great growth in the rat and often reaches the neck of the follicle. In its lower part it has a rich plexus of nerves and blood vessels. While the above account of the sheath layers is generally true, they have a far greater thickness in some places than in others and merge into indistinctness both in the region of the papilla and in the conical body.

From this description of its development it will be seen that the tactile hair of mammals is similar to the ordinary hair, from which it differs only in its greater development and higher specialization. It is a cell structure arising by differentiation from the epidermal cutaneous cell layers and thus is essentially unlike the invertebrate organs which resemble it but which are formed by a chitinous secretion.

The superior and inferior enlargements in the follicle have hitherto attracted much attention. They are the result of a thickening in the Malpighian layer of the outer root sheath. These cells not only multiply so as to form a greater number of layers but the cells themselves increase greatly in size. This growth

may be due to the augmented vascular supply to this place or to the stimulating effect of the many nerves which have their terminations here. The outer layers of these cells lie upon the leaf-like terminal expansions of the nerves and are connected in some intimate way with their functioning.

The ringwulst grows out of the root sheath. It appears as a somewhat oval shaped body projecting into the ring sinus. In prepared sections it is always much shrunken, but in its expanded state it must nearly fill the space between the walls of the diverticulum in which it lies (fig. 6). It is composed of connective tissue fibers (fig. 7)—fibro-hyalin in the rat—which enclose in their meshes great, clear, round, transparent cells with pale nuclei. It is penetrated in every part by loops of capillaries and by delicate varicose nerves. The nerves are not only distributed to the organ itself but also many of the larger nerve trunks which terminate in the superior enlargement of the root sheath perforate the substance of this body on their way thither.

The conical body is the name given to an enlargement of the root sheaths above the sebaceous gland. It is really no separate structure, but here the follicle layers are fused and it is simply that portion of the follicle walls nearest the surface of the skin. Many nerves and blood vessels pass through it on their way to the lower parts of the follicle. It has a muscular formation which probably indicates its chief function (fig. 1).

The lower sinus is sometimes called the cavernous sinus or sometimes this part of the follicle is named from the tissue which fills it, the spongiose body. The space is crossed by delicate cordons of connective tissue which enclose lacunal cavities. These are found filled with blood if the animal be killed without bleeding. The amount of blood is so great that good stained sections are impossible to obtain unless the blood has been drawn. The connective tissue network extends as far as the ringwulst and upon it and among it are found fine nerve fibers and small blood vessels. It is so tender of fiber that in injecting or in sectioning it is inevitably destroyed and one sees, usually, only broken fragments with here and there a few intact interlacing strands from which one must imagine the whole (fig. 8). The

upper part of this sinus when filled is practically closed by the ringwulst which separates it from the entirely free space above, the ring sinus.

Arteries and veins. The fibrous sheath is supplied with the ordinary nutritive blood vessels. These are particularly numerous in the middle layer of the sheath.

The large follicle artery enters with the main nerve at about the lower third of the sheath. It here divides and sends a branch to the lower part of the follicle and several branches upward which in turn divide and encircle the follicle longitudinally as far as the ring sinus into which they empty from below. To do so they run close to the walls of the follicle between them and the ringwulst to which they give many capillary branches.

Besides this main artery there are other smaller ones which come from the subcutaneous plexus and penetrating the walls of the dermal sheath debouch into the sinus at about the same place as the others.

More numerous than these vessels from below, however, are those which come down from the upper vascular plexus which lies just below the corium papillae. They accompany the nerves for the nerve ring about the neck of the follicle and open into the roof of the sinus just below the ring. In good preparations there may be seen a series of such perforations encircling the follicle in the constriction of the walls which form this roof.

I never saw any large veins in the follicle and think the venous outlets are, as Bonnet ('78) and Dietl ('73) describe them, through the outer coats of the bulb emptying into the skin veins.

Muscles. There is a veritable network of muscles surrounding the follicle. For the most part these are skin muscles and they help to obscure the real muscular attachments of the bulb.

There are longitudinal muscle fibers running down from the surface of the skin which have a membranous attachment to the walls of the follicle. It is also enclosed by horizontal bands of fibers so that it shares in the general skin musculature. Besides these there are long tendinous cords apparently forming part of the walls of the follicle which run deep down into the subcutaneous tissue and firmly anchor the follicle below. From the upper part

of the walls of one follicle muscle fibers run to the lower part of another in the same series so that any movement is a general movement. These may lay the hair back. They are all striped fibers (fig. 5).

Besides this connection of the follicles, there is a flat muscle band of fibers which surrounds the follicle on three sides. It originates in the wall of one follicle, runs around it and is inserted at the same level in the walls of another follicle. This muscle is well seen in a horizontal section (fig. 4). The nerve enters, as Bonnet ('78) says, on the muscle free side. These muscles probably have to do with the constant quivering movement of the hairs.

About the neck of the follicle are both longitudinal and horizontal contractile fibers and also about the neck running horizontally are plain muscle fibers which lie just below the nerve ring.

The conical body also has a muscular structure. The contraction of this organ opens the mouth of the follicle and permits a free vibration of the hair for a considerable depth in the follicle just as the contraction of the smooth fibers about the neck closes the walls about the hair and dampens the vibrations.

II. HISTORICAL

Although the tactile hair has been the object of many and extended studies, there are still histological and functional questions unsolved. The great size of its follicle tempts the unwary investigator; those who study the skin upon which it is found can not ignore it; and to the complexity of its structure each new histological method and stain must be applied.

One is struck by the contradictory statements made in these reports, particularly in the earlier ones; but the differences have several explanations. These hairs have such a general similarity to the body hairs and to the hairs of the head that the facts found true of the one have been carried over, wrongly, to the other. Then there is the false assumption that the tactile hairs of all animals agree in structure. Dietl ('73) has shown, and his statements have been confirmed by others, that there are two kinds

of sinus hairs, those with a ringwulst and a ring sinus, as in the cat and rat, and those without the ringwulst with only one sinus and that filled with trabeculae, as in the horse, cow, etc. Still another cause of the apparent difference between authors is the matter of terminology, of inexact definitions of particular histological parts. This causes considerable confusion, for example in determining in which of the layers of the follicle the nerve endings are found. Some who speak of the outer sheath mean merely the fibrous part beyond the blood sinus, but others mean the coats up to and including the glassy layer within the blood sinus. The glassy layer has been called a dermal and an epidermal structure; it has been thought by some to consist of one homogenous layer and by others to be made up of two layers; and still another view recognizes in it two layers of different origin, a dermal and an epidermal. For this reason when a writer says that the nerves go through the outer root sheath or through the dermal sheath one must stop to inquire what is meant.

The literature of this form goes back to Haller in the eighteenth century and the subject has been a most fruitful one ever since. Bonnet made an exhaustive study of the literature in 1878, Botezat in 1897, Szymonowicz ('09) gives some of the more recent references, and a rather extensive bibliography will be found in connection with the general bibliography on hair given by Friedenthal ('08). This paper therefore will not attempt to deal with the historical side of the question in any thorough way.

Gegenbaur ('51) should be mentioned, who studied the tactile hairs of nine mammals, and Leydig ('59) who secured specimens of skin from every mammalian family and gives a wealth of detail considering the methods and means at his disposal, and Odenius ('66) who thought that the development of the ringwulst had to do with nocturnal habits. Ten years later Merkel ('76) found the touch cells in this organ and about the same time Ranvier ('75) described the menisques. Bonnet made one of the most complete studies. He devotes twenty-eight pages to a description of the innervation of this hair. Retzius published a series of articles in 1892, 1893 and 1894 on the subject and

Ostroumow a good paper in 1895. The same year Messenger had a very brief article. Botezat put out two careful studies in 1897 and 1902, while some of the more recent contributions are those of Szymonowicz ('09), and Tello ('05).

In the limits of this paper it is impossible to do more than mention a few of the many studies, but some of them will be referred to for matters of detail in the discussion which follows.

III. METHODS OF STUDY

The grosser structure of the nerve supply of the tactile hair may all be seen in a careful dissection. After hardening the head in 10 per cent formaline for a few days, if the skin be cut posterior to the vibrissae and slipped down over the snout until the hairs are reached, the follicles appear, as they are torn away from the subcutaneous tissue, projecting from the epidermis. The branches of the facial nerve are in sight lying over the strong masseter muscle. If the anterior portion of this muscle be now carefully cut away, the sensory nerve will be exposed as it lies directly upon the bone. This nerve may now be followed even to the divisions entering each separate follicle.

The microscopic studies in this work were made from sections of degenerated nerves stained by the Marchi method, normal tissue stained with osmic acid, by Cajal's silver nitrate method and by Bielschowsky's variation of the same; but the intra-vitam methylen blue method proved the most satisfactory of all. The process adopted with this stain was that described by J. G. Wilson in *The Anatomical Record*, 1910 (vol. 4, no. 7).

IV. INNERVATION

General description. The large sensory portion of the fifth nerve emerges from the Gasserian ganglion in three divisions, of which we are here only concerned with the second, the superior maxillary. This passes through the fissura speno-orbitalis and then runs along in the infra-orbital groove and while there branches are given off to various parts of the mouth, pharynx and nostrils; but the infra-orbital branch in which we are inter-

ested is the terminal one. It passes through the infra-orbital foramen and lying close against the maxilla and pre-maxilla bones runs forward until just beneath the tactile hairs.

In sections stained with osmic acid the nerve is seen to be a typical cutaneous nerve consisting of many bundles of very different sizes and containing large and small fibers, fibers which stain very black, paler fibers and fibers which are not stained at all. It is a very large nerve in comparison with the branch of the seventh which lies superficial to it. Even as it emerges from the ganglion it has the form of a large flattened band and its branches look like mere threads beside the main trunk. It is estimated that this trunk as it leaves the infra-orbital foramen contains from 15,000 to 20,000 fibers. Its great size corresponds to the functional relations of the mouth parts which it serves. In animals like the elephant, tapir, etc., or animals with strong tactile hairs, its proportions are increased enormously.

It often divides into ten or a dozen branches before passing through the foramen, which is very much enlarged in many rodents. The inferior portion of the anterior maxillary part of the zygomatic arch is thin and flattened in rats, forming a vertical fissure through which the infra-orbital nerve passes. The superior part of the fissure is a rounded depression which at first glance might be taken for the orbit itself.

Soon after leaving the foramen this nerve anastomoses with the infra-orbital branch of the facial, which is the motor nerve to the same region. The fibers of the trunk thus formed are distributed to the skin of the upper lip and nose, but about half of them go to the tactile hairs of which there are between forty and fifty on each upper lip (fig. 3). These are arranged in six rows with from five to nine hairs in each row, and there are often 150 or more large medullated fibers in the nerve bundle entering a single follicle of a large tactile hair. Usually, just before reaching the root of the hair the nerve divides in two parts which penetrate the dermal sheath at the level of the lower third. One part turns back, both divide many times and together they encircle the follicle in a sort of palisade of longitudinally running fibers. The further course of these fibers will be followed later (fig. 9).

The dermal plexus and the plexus of the outer root sheath. All over the dermal sheath of the follicle is a plexus of fine varicose fibers and a very similar one is seen on the surface of the outer root sheath. As one looks down on the follicle it appears to be covered with a fine network of nerves. It is difficult to say whether the fibers really anastomose or not, but in many cases where they can be followed they seem to lie over or under one another. They grow finer and finer with repeated divisions and when the end can be seen it is usually a simple fiber, or what is more common the fibril ends with one of the varicosities with which, as has been said before, it is studded. The fibers have very much the appearance of sympathetic fibers and some of them can be seen branching from nerves which accompany arteries. Botezat's opinion ('07) that these plexuses have to do with the nourishment of the follicle seems the true one. Since our interest is in the sensory nerve, these will not be discussed further and no attempt will be made to describe the motor nerves which may be seen going to the muscles of the follicle.

Nerves on trabeculae. Of the same nature are the nerves in the connective tissue strands of the lower sinus. The trabeculae which fill the large cavity of the blood sinus below the ringwulst furnish bridges for crossing fibers (fig. 8). Some of these surround the walls of the arteries which they accompany and others resemble these so much that, though their immediate connection with any blood vessel cannot be seen, one may infer them to be from the sympathetic system. Still others detach themselves from the trunk of the main nerve coming from below and cross singly on these pathways. The latter are for the most part sensory nerves destined for the lower part of the follicle and immediately turn back in that direction.

Nerves in the papilla. There are nerves forming a rich plexus within the papilla. They are usually varicose and sometimes can be seen running with the arteries. They often reach a considerable height in the medulla of the hair (fig. 10).

Bonnet was not the only one who thought the papilla without nerves ('78, p. 390), but Retzius ('92-'96), Orru ('94) and others described nerves in this place. Ostroumow thought

them all vaso-motor but Botezat ('97) denied this. They looked to him like the intra-gemmal fibers of the touch cells and he also described thickenings not unlike the knobs of the intra-epithelial nerves. He thought, therefore, some of them might be sensory.

From the position of the papilla and the little likelihood that the movements of the hair can be very effective here, as well as from the appearance of the fibers themselves, we believe these nerves to be comparable to those which may be found in any vascular structure and to be non-sensory in character. They come to the papilla in many little fibers from the subcutaneous tissue below and no connection can be seen with the large sensory trunk which enters the side of the follicle.

Sensory nerve. The great nerve which we have described before pierces the dermal sheath together with the main artery at about the lower third of the follicle (figs. 1 and 9). Here a few branches which serve the lower part of the organ break away from the rest, but the main portion of the nerve divides in two, one part turns to the opposite side, both divide and redivide and when they have crossed the sinus, surround the follicle with rows of parallel, ascending bundles of medullated fibers many of which may be distinctly traced to the constriction at the neck of the follicle. These bundles are not entirely separate, for fibers can constantly be seen detaching themselves from one bundle to join another so that it looks as though the follicle rested in a coarse cup-like meshwork of bundles of heavily medullated fibers. This is the outer plexus of the follicle proper and is most pronounced over the superior swelling of the root-sheath. It must not be forgotten that all along the course of these bundles, fibers which terminate at different levels are continually separating off; but the main trunks are of such size and stain so heavily that they are the most noticeable feature of the whole structure. As they come to the follicle and rest thus upon it they seem to lie embedded in a colorless substance which is probably the gelatinous endoneurium of the nerve trunk. Fibers which serve the upper part of the follicle bend outward at the region of the ring-wulst and pass up through it. It is the bending of these nerves outward which emphasizes the swelling of the root sheath here.

For the most part these nerves terminate in a one-layer mantle of touch cells all over the follicle in the Malpighian layer of the outer root sheath, but the endings are the largest and most characteristically developed over the superior swelling of the sheath (*a*, fig. 9). The large nerves preserve their myelin almost to the very end, when they suddenly go over into disc-like expansions of various sizes—leaf-like endings with thread-like stalks. The whole appearance is as if the sheath were flattened out very thin to furnish a support for the intertwined neuro-fibrils (fig. 11). At times there is just one of these leaf-like endings, again there may be two, three or four. When there is more than one, each member is connected with the preceding by a deeply stained fiber which arises from the corner or tip of the expansion and the whole series has a somewhat definite arrangement. I have often seen follicles which looked as if they were surrounded by horizontal or oblique running bands of these menisques. They have been well described by Cajal ('09, p. 474):

They show in the interior a fine network of neurofibrils separated by an abundant uncolored neuroplasm. This network is of the same composition as that of the body of the nerve cells. We find first, the large neurofibrils frequently bending and forming the framework of the termination and second, pale fine fibrils which bind together all the others. A large number of menisques are supplied by one nerve fiber.

The cells between which or under which these menisques lie are large ovoid cells, probably modified Malpighian cells of the outer root sheath. There is no connection to be seen between the cell and the fibrillar plexus of the menisques. What has happened is this: The nerve has lost its myelin and one of the fine fibers described before has pierced the glassy layer and expanded within under one of the large cells found here. The glassy layer is very thin over the superior swelling and disappears above it. As it is very dense and hard to perforate elsewhere, the greater number and size of the endings found at this place is accounted for. Yet fibers do push through at lower levels also.

There has been a great deal of discussion as to the relative position of these menisques with regard to the cell body and to the axis of the hair. In the rat there seems to be no one char-

acteristic position. In the superior root swelling where they attain their greatest size they usually lie vertical to the long axis of the hair and beneath the cell body. The flattened surface of the menisique is somewhat parallel to the surface of the skin. Near the glassy layer, however, the position is almost at right angles to the surface of the skin and parallel to the long axis of the hair. This is due no doubt to the resisting power of this layer. In the lower portions of the follicle—for these endings cover the whole of the follicle contrary to the opinion of many of those who made the earlier studies of the endings—they are more varied in position but may often be seen parallel to the axis of the hair shaft. Ranvier ('75) first described these structures truly and recognized them as the touch cells which Merkel ('76) had previously found in other parts of the skin. Subsequent investigations have shown that the fiber does not end in the cells, as Merkel first thought. It is generally believed now that this cell is simply a modified epithelial cell which may serve as a protective cushion for the nerve expansion and possibly help to increase or modify the pressure stimulus.

Besides the nerves which end in this way, fibers leave the main bundle the whole length of the follicle, run to the glassy layer and arborize upon its surface or end in free or flattened spatulate endings, so that the entire outer surface of the glassy layer is covered with a fine nerve plexus. Among these fibers are some which penetrate the glassy layer and end between the epithelial cells of the outer root sheath. Tello ('05) and Ostroumow ('95, p. 914) say that these fibers are of a different order from those which end in menisques within the glassy layer. That they usually appear smaller and not so heavily medulated, at least not so deeply stained, is true, but that they are different in origin or function seems doubtful. Among those fibers which end in menisques occasionally one is found which forms several small menisques before piercing the glassy layer; again from menisques within the glassy layer go off little fibrils which run between the cells of the outermost layer of the outer root sheath and end intra-epithelially or arborize about these cells, as Merkel first described; but besides these one may see at times nerves which

cross the glassy layer from *within* and form a small plexus *without* the layer in the same fashion and one then finds the leaf-like expansions both within and without the layer from the same fiber. Thus there can be discerned little difference save in development between the two kinds of fibers (fig. 12).

The nerve ring. To the neck of the follicle come also nerves which take part in the formation of the dermal plexus (fig. 13 and fig. 1). Some of them are diverted to the follicle before joining to form the plexus, some send a long branch downward to the follicle and another up to the plexus, others come out from the plexus itself, often in groups of from three to a half dozen fibers among which may be seen deeply stained medullated fibers, pale and varicose fibers. These nerves are much smaller than those of the large sensory trunk which enters the lower part of the follicle but on the other hand there are many of them and they approach the mouth of the follicle from all sides. With them are blood vessels from the vascular plexus and many both of the nerves and blood vessels go to serve the very large sebaceous glands of this structure. The rest run to the region just above the ringwulst, that is just below the sebaceous glands, and here most of them lose their myelin and encircle the hair at the level of the glassy layer.

The nerve ring is deeper than the longitudinal ascending fibers, many of which pass over it. The nerves which form it often divide once before beginning their circuit and the two branches sometimes take opposite directions, but there is no further division until they have nearly or quite completed the circle; then they ascend and break up into what looks like an arborization in the conical body. From this brush of fibers whose ends are cut across in a longitudinal section may often be seen fibers running up to the surface of the skin again. I have never seen the longitudinal fibers take any part in the formation of the nerve ring.

The course here described is for the strong deeply medullated fibers. As these run down from the surface they are accompanied by paler fibers and varicose fibers, as has been said, but their course I could not follow after they were lost in the intricate ring plexus.

A brief review of some of the positions taken as to this nerve ring band may be useful. Leydig ('59, p. 390) saw the ring but others after him denied its existence. Bonnet ('78, p. 366) said the fibers came from the outer plexus about the neck of the follicle and reached the ring in some unknown way, but Messenger ('90, p. 401) thought it was composed of fibers from the nerve trunk entering from below and Botezat declared that he saw fibers in the ring from both sources ('03). Most authors agree that these fibers end on the outer surface of the glassy layer, but Ranvier says that they go through this layer ('75). As to their mode of termination, few have been able to describe them. Ranvier said they end in spatules ('75, p. 915); Botezat ('02) described them as simple endings, thickened or flattened forms and Szymonowicz ('09, p. 622) as forked endings.

It is very easy to confuse the terminations of these fibers with those of the branches of the longitudinal fibers which end many of them here on the glassy layer. The fibers of the ring are very fine and very easily broken in the preparations, so that it is exceedingly difficult to be sure as to free endings or endings in varicosities. Sometimes I have thought that I saw connections between these delicate fibers and some plain muscle fibers which surround the neck of the follicle but farther than this I can say nothing about the endings here. I think the significance of this ring has been over-estimated and for reasons which follow do not agree with Messenger who says, "The annular nerve band is so situated that when the pulvinus is not turgid tactile impulses are little felt but when it is turgid the slightest impact produces a marked effect on the nerves surrounding the hair" ('05, p. 401). Neither do I agree with Bonnet who thinks that the hypothesis of a real intensification of power of perception through this nerve ring is perhaps justified. Odenius' ('66) assertion that the ring is confined to nocturnal animals has been refuted by others.

Ringwulst. The structure of the ringwulst has been described before. It springs out just beneath the superior swelling so that all the large sensory fibers which terminate above it must pass through it. Many of these nerves run close to the walls of the follicle but others bend outward and pass through the ringwulst

midway striking the walls of the superior enlargement above. It has been said by Botezat, Szymonowicz and others that the ringwulst only surrounds the follicle from two-thirds to three-fourths of the circumference, but in the white rat this is not so. The tissue is very fragile and in most of the methods of preparation it is badly torn, but with silver nitrate and osmic acid the structure is often whole and entirely surrounds the follicle. It shrinks much in staining, in length as well as in width, which may account for the gap one often sees in the circumference.

This very tender organ consists of almost transparent connective tissue fibers which enclose large pale nucleated cells. In the rat, in a longitudinal section, the structure has somewhat the shape of a lung which comes out by a stalk from the follicle walls and hangs suspended in the cavity of the sinus. It is traversed in all parts by loops of capillaries which are unusually large for the size of the organ (fig. 7).

As one looks down upon it in well prepared sections it seems covered with fine varicose fibers which run from the base out to the periphery and end with a few fine branches or with small varicosities (fig. 6). Whether these are sensory or vaso-motor, I have no way of knowing.

The ringwulst is not found in all animals according to Bonnet and others. The horse, cattle and swine are without it while in carnivora and rodents it is fully developed. Botezat ('97, p. 144) shows, however, that some animals, as the swine, have two kinds of tactile hairs; those with and those without a ringwulst and a ring sinus. Where there is no ringwulst as in the horse, there are often, Bonnet ('78, p. 348) says, similar thickenings on the walls of the trabeculae near the conical body or else where several such walls come together. These thickenings may serve the same purpose as the ringwulst, whatever that may be.

V. COMPARATIVE ANATOMY

Hair is a mammalian characteristic, but the tactile hair is probably phylogenetically the first to appear and when all other hair is lost, as in the whale, a few of these still persist about the

mouth parts. Human hair begins to develop around the cutaneous orifices between the first and third months. Krause saw tactile hairs earlier than any other hairs in the mole foetus of 9.5 mm., and in rabbits in the second half of the embryological period they were completely keratinized, the follicle showed the two swellings and contained many blood vessels which corresponded to the blood sinuses.

A number of investigators have said that the structure and innervation of the hair differed in young and adult animals but tissue which I have stained from the rat of a few days contained perfectly formed follicles with the usual mantle of touch cells and a nerve ring as well. The course of development of this mantle and nerve ring is worth our further consideration.

Merkel ('76) discovered some peculiar cells in the snout of pigs, round glistening cells in the inter-papillary spaces, the 'epithelial Einsenkungen' of the Germans, to which came terminal fibers ending in flattened discs about the cell or, as he then thought, within it. He concluded that these were ganglion cells. Later studies showed that there was no real connection of the fiber with the cell and also that these cells were not confined to the mouth parts of animals but were found on the cutaneous epithelial border of human skin, in tactile hairs and in other places. Szymonowicz, in a long series of articles, has shown that these are merely the usual epithelial cells whose differentiation has been caused by the coming of a nerve fiber and the formation of a fibrillar plexus about the cell.

Eimer ('94) studied in the snouts of animals a peculiar arrangement of cells in a cylindrical or hour-glass form. These have since been studied by Jobert ('72), Krause, Szymonowicz ('95), Botezat ('02) and others. The structure, which has been named Eimer's organ, lies in the same skin layers as the touch cell and to it come medullated nerves which lose their sheaths and run up between the cells as a central bundle of fibers whose lateral branches lie between the cells or as a cup-shaped plexus about them. These are evidently of the same nature as the touch cells of Merkel in the inter-papillary spaces and also of the touch cells of the tactile hair.

The embryological studies of Szymonowicz ('95) prove that these cells originally lay in a horizontal layer between the cutis and the epidermis but were pushed by the formation of the cutis papilla into the inter-papillary spaces and finally lay in groups over each other. Botezat ('97, p. 103) tells how in the invagination of the hair follicles these cells and the nerve endings are carried down and lie in the root sheath of the follicle in the same position which they originally occupied on the cutis border. He calls attention to the fact that if one were to fix a hair in one of these groups of cells, particularly in Eimer's organ, one would have a structure very similar to the hair follicle without the fibrous sheath. He mentions the likeness of the touch cells to the cells of Grandry's corpuscles and also to the platelets which Bethe describes in the tongue of the frog. Merkel found the touch cells in the region of the mouth or nasal openings of many mammals and apart from this part of the body chiefly in the un-haired portion of the skin. He also found them on the hard palate and the bills of birds, on the tail of the hedgehog and the vulva of swine. In man he said they are situated for the most part where there are no Meissner's corpuscles. There are many over the abdomen. Ranvier ('60) says there are more menisques in the finger tips than there are cells.

Enough has been said I think to show the very general distribution of these simple endings; of the functional importance as well as the particular distribution we will speak later.

The nerve ring may be explained as the position of the touch cells has been explained, as arising as a result of the process of invagination. In the infolding of the follicular layers the nerve plexus is carried down too for a short distance. The follicle finally breaks through it but in the subsequent enlargement of this organ, and great increase in diameter, the fibers of the plexus are stretched out in the so-called ring. The brush-like ends which are seen cut across in the conical body are a part of this same plexus and from these fibers may often be seen running up to the surface. These nerves in the hair then have no special function but are simply a distorted part of the common skin plexus.

All comparative study has shown that neither the size and development of the tactile hair follicle nor the nerve endings are proportional to the size of the animal. The rat, for instance, possesses a far more highly developed tactile hair than the horse or the ox or even the rabbit among the rodents. Leydig ('59) fifty years ago, noted that in the horse the follicle was smaller than in the dog or ox and the walls were thinner. In the polar bear he found only a slightly developed tactile hair with a small follicle and a sinus much more tender than a dog. The weasel and otter, on the contrary, have longer follicles with a greater ring sinus than the dog. The porcupine's follicle is egg shaped and unusually large, but the greatest development of all the trabecular filled sinuses is in the seal where it may be seen with the naked eye.

Of the investigations of the tactile hairs of the apes we shall mention only one, that of Frederick ('05). He says that in some apes the tactile hairs are so strong that they can be seen as such by the eye alone. On the upper lip are many longer hairs which are the longest and strongest laterally. As a rule the hairs are weaker on the under lip. On microscopic study it is seen that these hairs on the lips and in the supra-orbital regions are real tactile hairs. The follicle is enclosed from without by a fibrous capsule of connective tissue and between the external and internal lamina are cavernous spaces filled with blood. These extend from the mouths of the sebaceous glands to the papilla. Frequently they extend beneath the papilla, between it and the base of the follicle, the whole space being filled with a close mass of interpolated cavernous tissue. In none of the apes studied was there any differentiation between the ring sinus and the spongiform body; instead the whole cavernous space in its whole length was crossed by numerous closely radiating connective tissue bundles which connected the lamina externa with the lamina interna. He found the ring sinus lacking in apes, which in this respect are like hoofed animals.

Szymonowicz ('09) finds the endings described in the tactile hairs of other animals on the beard hairs of men. The nerve ring contains more medullated fibers than is usually found in

other animals and forms a rich plexus of medullated, unmedullated, pale and varicose fibers. The touch cells are similar to those of other animals and in a similar position. He thinks the beard hairs of men lie structurally between the usual body hairs without the root sheath swelling and the tactile hairs of other animals.

We will neglect entirely the discussion as to whether hairs originally developed from the scales of reptiles or fishes or from the Becher organs of amphibia, but there is one view of the course of their development that is not without interest for us. Many believe the theory which Botezat advances that the common body hairs are retrogressive, that the tactile hairs were primary in development but that as functional need changed the greater part lost their root sheaths and high degree of innervation and came to serve more and more as a mere body covering.

As to the higher development of these tactual organs in some animals than in others, Edinger ('08) calls attention to the large fiber tract leading from the nucleus of the trigeminus to the tuberculum olfactorium in birds and some other animals. He thinks the increase in size of this lobe in some instances due to the "importance of the beak which is innervated by the trigeminus" and "the extraordinary rich trigeminal supply about the mouth and in the tongue." Probably other fibers find a center in or about the anterior perforated space but Edinger's emphasis of the 'oral sense' has not been without value. We must recognize that the mouth parts of animals have a much more varied functional significance and are of far greater relative importance in them than in man.

VI. FUNCTION

The generally recognized character of the tactile hair is implied in its name. The purpose of such sense organs about the mouth has never been satisfactorily explained. They are not confined to nocturnal animals, as Odenius thought ('66), nor do they have chiefly to do with the size of openings.

In a long series of experiments with animals in open, elevated mazes and in problem boxes, some facts were established as to

the use of these tactile organs. The vibrissae were turned down and trailed along the edges of narrow supports or they brushed the sides of vertical walls or the path beneath as the animal ran. Animals deprived of them learned such problems less easily and had many more slips and falls. The inference was drawn that they furnished guiding sensations, sense of support, in locomotion, that they were intimately connected with equilibration and that their extreme mobility and sensitivity in rats was a partial compensation for poor vision. This was confirmed in part by observations of rats whose labyrinths had been destroyed. The tactile hairs seemed also to assist in determining the exact position of openings or turns and in the discrimination of inequalities of surface. Animals in which the sensory nerve to this region had been cut, and whose noses as well as vibrissae were insensitive, were unable to make this tactual discrimination. A careful record of the return of sensitivity to the vibrissae of these operated animals was kept for seven months. One of the most conspicuous features of this period was the trophic changes in the hairs. They curled, split, grew brittle, and finally broke off and at the end of seven months when the nerves were regenerating and sensibility was returning many animals had not half as many of these hairs, and of those present many were broken and imperfect. The wound healed without suppuration. In two weeks the animals were in perfect health. The sensory innervation of the follicle appears in some way to be the stimulus to, or condition for the growth and maintenance of the hairs by whose movements the nerve endings themselves are excited.

The complete details of the experimentation, the mode of operation and the tests for sensitivity are described in my monograph, *The Function of the Vibrissae in the Behavior of the White Rat* (Vincent '12).

The attempt to explain structure by physiological function has not been on the whole successful. Messenger ('05) bases his account of the haemostatic structure of the follicle on the absence of erector muscles; but there are muscles in sufficient number to account for all the movements of the hairs.

Poirier and Charpey ('07) give one of the best accounts of the way the organ functions:

When an excitation occurs which affects the tactile hair it produces first a motor reflex. Under the influence of the muscles the hair straightens itself again. In erecting it compresses the venous vessels in the blood sinus. These do not delay in distending themselves—and permit the blood again to enter. Also the nerve terminations are compressed between the hair and the blood sinus. They are thus stimulated and transmit to sensory and peripheral neurones the impressions which they receive.

There are many questions one would like to ask concerning the relation of structure to function here. The following account is as complete as we can make it at present. The hair is a powerful tactile organ. Its great innervation would imply this and it has already been discussed. There are other factors, however, which contribute to this end, among which we may mention first the area stimulated. Comparing the area of the hair shaft with the area of the invaginated inner follicle with its mantle of touch cells we find that the actual surface stimulated by the vibrations of the hair is 200 times that occupied by the hair itself on the surface of the skin. This of itself would be enough to justify our first point but there are still others. The hair whose delicate tips extend beyond and to the side of the head is a lever whose fixation in the follicle magnifies the effect produced by the slightest touch. Sherrington ('00) says:

The short hairs of the skin much enhance its tactual sensitivity. On 9 sq. mm. of skin from which the hairs had been shaved the liminal stimulus was found to be 36 mgms., whereas, on the same surface, before it was shaved, 2 mgms. was the liminal stimulus. The liminal stimulus for the touch spots about a short hair is three to twelve times greater than for the hair itself, i.e., the hair is three to twelve times more sensitive than the "spots." Each short hair is a lever, of which the long arm outside the skin acts at an advantage upon the touch organs at the root. The short hairs are probably the most sensitive tactual organs of the body.

The muscles of the conical body are such that by their contraction this hair is permitted to vibrate freely to the very depth of the follicle, while on the other hand contraction of the fibers

about the neck of the follicle will dampen the vibrations. These are probably under efficient reflex control.

Several reflex phenomena are the result of the nature of this stimulus and the mode of its application. Only vibratory or intermittent stimuli are the adequate stimuli for certain reflexes. According to Sherrington ('06) a scratch reflex which cannot be evoked by a single induction shock, or even two unless very intense, can be produced by a series of subliminal stimuli (44 in one instance) through spinal power of summation. The same reflex may be produced by a rub, prick or pull upon a hair; but as he says, "there is nothing to show that these stimuli, though brief, are really simple and not essentially multiple."

The following statement is taken from von Frey ('96, p. 238): "The significance of the hair is less in the perception of passive weight than in the perception of fleeting impressions, or moving stimuli. . . . The hair functions as a lever. The nerve excitation is not the effect of pressure but the expression of a vibratory movement." Perhaps such vibratory stimuli are adequate because they lead to summation.

A vibratory stimulus besides its summing power has, in the nature of things, a tendency to prolong the initial stimulus.

Another condition which increases the tactual power of these organs is the muscular connection of one follicle with another, so that there is a general spread of excitation, irradiation of stimulation over a comparatively wide area.

The erectile tissue and blood sinuses are in a sense necessary to the free vibration of the hair and stimulation of the end organs. This free vibration could not occur if the hair shaft were fixed in a firm unyielding tissue. They may also, as others have pointed out, serve to increase or modify the pressure, but as the pressure is not directly exerted through the skin the modification must be brought about through the varying resistance offered to the excursions of the hair shaft itself. The erection of this vascular tissue may also, as Dr. Herrick has suggested, lower the nervous threshold as compared with that of the flaccid state and an efficient reflex control be brought about in this way. Those who believe that all sensation is chemical in origin may see in this

arrangement for flooding so richly an innervated region with blood some basis for such conclusions.

One factor has yet to be mentioned—The vibrissae are, in a way, secondary sexual organs. The vibrissae of the males are larger and stronger. This difference is not great in rodents but sufficient to attract attention and cannot be explained by greater health and vigor on the part of the male.

Darwin believed that the beard was first acquired as an ornament, but Cunningham thinks that it arose in response to stimulus caused by attacks about mouth and throat ('99, p. 41). In animals where stroking about the head and mouth is a part of courtship, these organs may contribute to the excitement. While we cannot but admit that some secondary sexual characters seem more intimately connected with sex functions than others, it does seem probable that vascular structures such as we have described in these hair follicles may share or in some way contribute to the emotional excitement. The *sensory quale* arising from such reflex excitation might serve to enhance such emotion. At any rate we must face the fact that the abundant vascular supply may possess an affective or emotional significance and not be entirely connected with a sensory, tactile function.

VII. SUMMARY

The follicle of this tactile hair consists of invaginated skin layers which form the outer and inner root sheath. It has besides these a dermal sheath between whose layers are large blood sinuses. The lower sinus is filled with erectile tissue and separated from the upper by an outgrowth of the root sheath called the ring-wulst. There are striped muscles enough to account for all the movements of the hair. The follicle has a distinct and extensive blood supply. It has two innervations. A large nerve bundle from the infra-orbital branch of the trigeminus pierces the dermal sheath in the lower part of the organ, spreads out over the inner follicle in a heavy plexus and terminates chiefly in a mantle of touch cells in the outer root sheath all over the follicle. From the dermal plexus of the skin branches run down and form a nerve ring about the neck of the follicle. Many of these fibers are also

from the trigeminus, as studies in degeneration show. The touch cells are similar to those which Merkel found on the cutis border and have invaginated with the skin layers and reached a higher development here. They are found especially well developed in the mouth parts of many animals and are described by Szymonowicz on the beard hairs of men. The size of the follicle and the richness of its innervation do not depend on the size of the animal but upon the tactual functional significance of the mouth parts. Histological and structural studies show that the tactile hair is a powerful organ of touch and that this is due:

- (a) To its great innervation.
- (b) To the increase in the area stimulated.
- (c) To the leverage which magnifies the stimulus.
- (d) To the vibratory nature of the stimulus, which is the only adequate stimulus for some reflexes, which summates subliminal stimuli, which prolongs the initial stimulus.
- (e) To its muscular connection, which transmits stimulus over large areas.
- (f) To its haemostatic apparatus, which permits free vibration of the hair to the depth of the follicle, which may increase or modify pressure, which may raise or lower the nervous threshold, and which may possibly have some chemical significance.

Experimental studies with living animals show that:

- (a) It is an aid in locomotion.
- (b) It functions in equilibration.
- (c) It aids in determining nearness or position of edges or corners.
- (d) It is an aid in the discrimination of inequalities of surface.
- (e) It is a supplement to poor vision.

This work was done under the direction of Prof. J. G. Wilson in the Otological Department of the Northwestern University Medical School. The expense of the research was in part defrayed by a grant from the Patten fund.

I should like to acknowledge my indebtedness to Professor Wilson for constant advice, to Professor Herrick of the University of Chicago for suggestions during the course of the investigation and to Miss Hill for her careful drawings.

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PLATE 1

EXPLANATION OF FIGURES

1 Longitudinal section of follicle. This follicle was drawn from a Cajal silver preparation but some features of the nerves and arteries have been added from other preparations. It shows: *a*, nerve from dermal plexus running down to form the nerve ring; *b*, conical body; *c*, sebaceous gland; *d*, artery entering ring sinus; *e*, ring sinus; *f*, nerve ring; *g*, dermal sheath; *h*, ringwulst; *i*, root sheath; *j*, cavernous sinus with trabeculae; *k*, main sensory nerve from below; *l*, large artery entering with nerve; *m*, papilla.

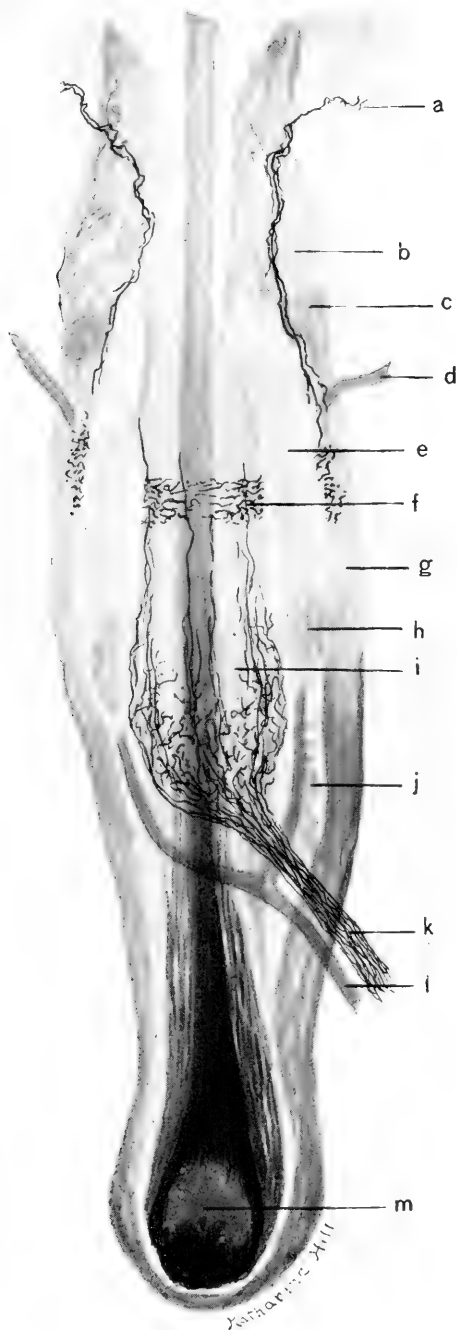


PLATE 2

EXPLANATION OF FIGURE

2 Tip of follicle showing invaginated skin layers; *a*, hair shaft; *b*, cavernous sinus filled with trabeculae and clotted blood; *c*, dermal sheath; *d*, inner layer of dermal sheath; *e*, glassy layer; *f*, outer root sheath; *g*, inner root sheath; *h*, invaginated outer root sheath—this forms the medulla of the hair shaft; *i*, papilla. $\times 100$.

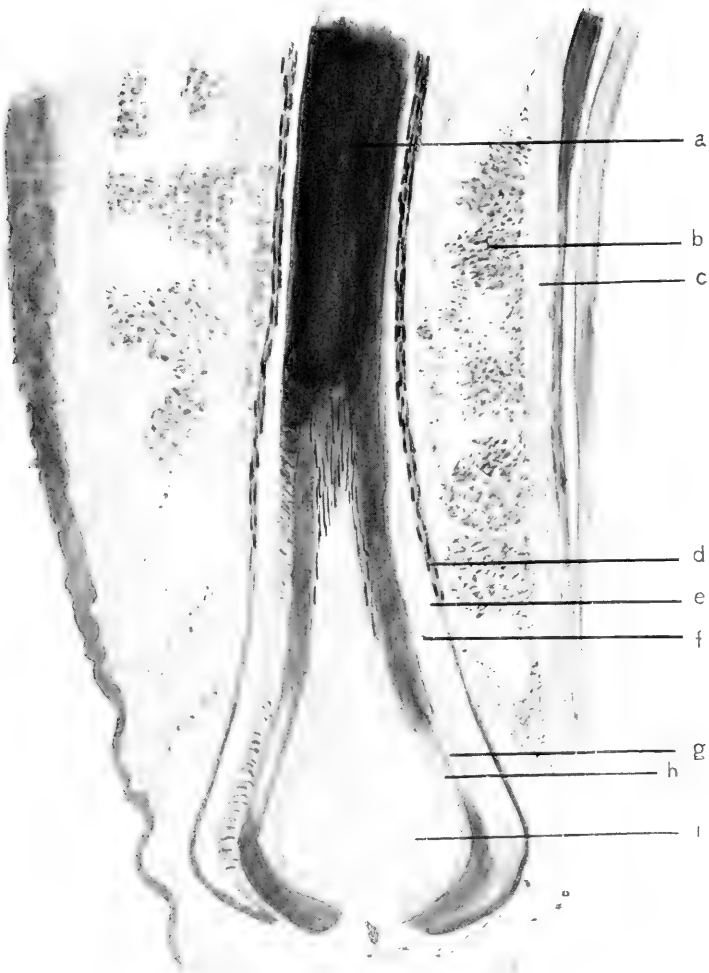


PLATE 3

EXPLANATION OF FIGURES

3 Dissection showing: *a*, infra-orbital nerve running out to the tactile hair follicles and also its anastomosis with the facial; *b*, follicles of the tactile hairs; *c*, facial nerve.

4 Horizontal section of follicle just above papilla showing muscles. This also shows the sensory nerve just entering. It has already divided. A cross section of the inner follicle may be seen and the cavernous sinus with the crossing trabeculae. $\times 27.5$.

5 Musculature of follicle. $\times 27.5$.

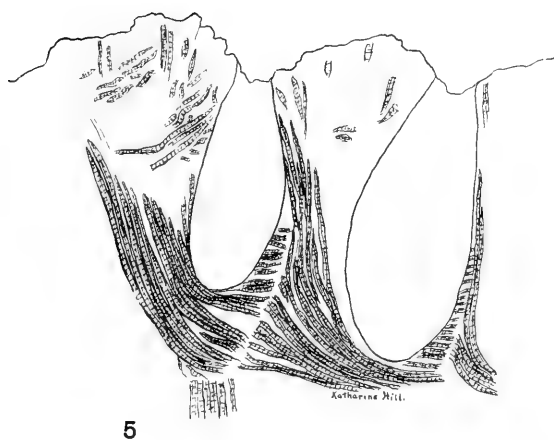
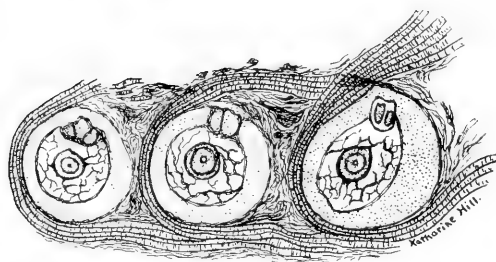
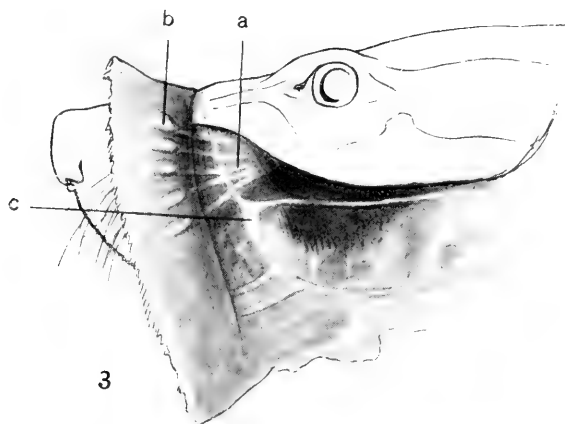
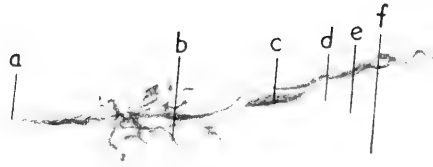


PLATE 4

EXPLANATION OF FIGURES

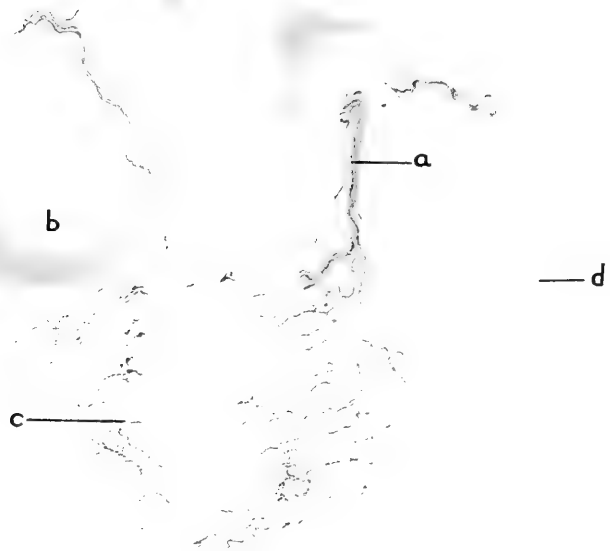
- 6 Ringwulst showing varicose nerves. $\times 140$.
7 Ringwulst showing detail of structure. $\times 750$.
8 Detail of trabeculae in lower sinus showing nerves. $\times 750$.
9 Main sensory nerve entering from below; *a*, superior swelling of root sheath where the characteristic endings seen in figure 11 have their greatest development; *b*, ringwulst; *c*, one of the chief divisions of the nerve. $\times 140$.
10 Nerve in papilla. $\times 100$.
11 Tactile menisques. Over each of these leaf-like expansions lies a large round cell which does not show with this stain. $\times 533$.
12 Intra-epithelial endings; *a*, nerve fiber running outside the glassy layer; *b*, intra-epithelial endings in outermost layer of the outer root sheath from the same fiber; *c*, menisque outside the glassy layer; *d*, glassy layer; *e*, epithelial cells of outer root sheath; *f*, outer root sheath. $\times 533$.
13 Nerve ring about neck of follicle; *a*, nerves from dermal plexus running down to form nerve ring; *b*, sebaceous gland; *c*, nerve ring. $\times 140$.



12



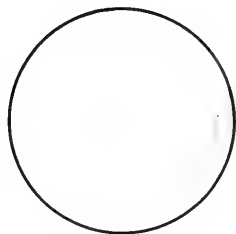
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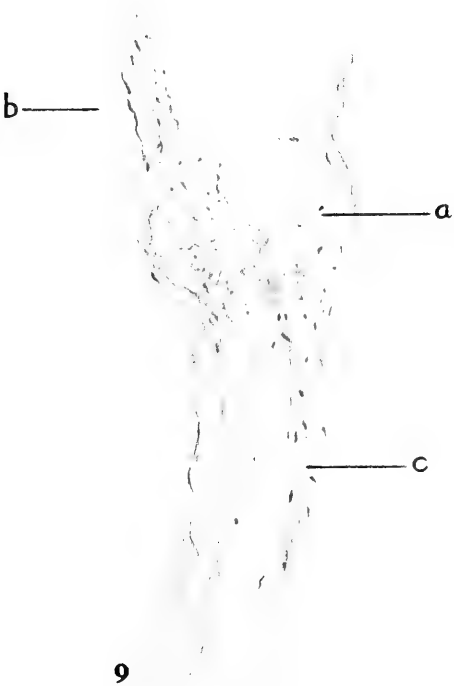
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11



PRENATAL GROWTH OF THE HUMAN SPINAL CORD

MAX MAYO MILLER

From the Anatomical Laboratory, University of Missouri

TWELVE FIGURES

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INTRODUCTION

In human embryology the changes in form and the histological differentiation in the cellular elements of the spinal cord have been studied very carefully. But as yet little has been done on the absolute and relative prenatal growth of the cord as a whole and

of its various regions and parts. To throw light upon this matter the present study was undertaken. The data here presented include: First, the absolute and relative growth of the spinal cord in its entirety; second, the absolute and relative amounts and the rate of growth of the different regions of the cord; and third, the absolute and relative amounts and the rate of growth of the gray matter, of the white matter and of the ependyma with the canal. This investigation was carried on in the Anatomical Laboratory of the University of Missouri, under the direction of Prof. C. M. Jackson, to whom I am also indebted for the use of his collection of human embryos.

MATERIAL AND METHODS

The material used consisted of the following embryos: 11 mm. (No. 60, fifth week), 17 mm. (No. 58, sixth week), 31 mm. (No. 57, eighth week), 65 mm. (No. 55, twelfth week), and 150 mm. (No. 54, five months). The lengths are all crown-rump measurements. The ages are only approximate and all conclusions referring to them are, therefore, subject to more or less uncertainty.

The embryos had been prepared by the following methods: No. 60 (♀?) was fixed and hardened in alcohol, stained in bulk in alum-cochineal, embedded in paraffin, and cut into transverse serial sections, 20μ thick. No. 58 (♀) was fixed in formalin, hardened in alcohol, decalcified in acid-alcohol, stained in bulk in alum-cochineal, embedded in paraffin, and cut into transverse serial sections, 20μ thick. No. 57 (♀) was fixed in formalin, hardened in alcohol, decalcified in acid-alcohol, stained in bulk in alum-cochineal, embedded in paraffin, and cut into transverse serial sections, 20μ thick. No. 55 (♂) was fixed in formalin, hardened in alcohol, decalcified in 1 per cent HCl in 70 per cent alcohol, stained in bulk in alum-cochineal, embedded in paraffin, and cut into transverse serial sections, 50μ thick. No. 54 (♀) was fixed in formalin, decalcified in 2 per cent nitric acid in 70 per cent alcohol, embedded in celloidin, cut into transverse serial sections, 100μ thick, and stained with alum-haematoxylin. This material is all in good condition, especially the younger embryos.

Sections of these embryos were magnified by means of an Edinger projection apparatus (E. Leitz-Wetzlar) and outline drawings made of cross-sections of the spinal cords. In the case of the 11 mm., 17 mm., 31 mm., and 65 mm. embryos, every fourth section was drawn, while in the embryo of 150 mm. only every tenth section was used. In exceptional cases where the section to be drawn was torn or distorted, the adjacent section was drawn instead. The magnification used was 50 diameters, which corresponds to a magnification of 2500 times in cross-sectional area.

The areas of these drawings were measured with a planimeter (Coradi). This instrument on being tested showed an error of less than 0.25 per cent. Two entirely independent readings were made in each case and the average used in order to minimize the error. The percentages of the gray matter (anterior and posterior horns), of the white matter (anterior, lateral, and posterior columns), and of the endyma with the canal were first calculated from the original readings. The original readings were then reduced to their actual size from which again the percentages of the various parts were calculated. This gave a check on the accuracy of the calculation. Since the thickness of the sections was known, it was easy to calculate the total volumes of the cords and their various parts.

The first section which showed filaments of the first pair of spinal nerves was taken as the upper level of the spinal cord. This, however, was not always the exact upper level, on account of the obliquity of the section caused by the normal curvature of the younger embryos. This error is in most cases not large enough to interfere seriously with the general results, but should be borne in mind.

The length of a segment was determined by taking all sections between the uppermost point of attachment of a nerve to the cord and the corresponding point of the next pair of nerves caudal to the first. Since the thickness of the sections was known, the lengths and volumes of the various segments were readily calculated. Owing to the curvature of the younger embryos it was impossible in these to obtain the exact length of the upper and the

lower segments. This, however, did not interfere with obtaining accurately the total volume of the cord and of the various regions. In the 65 mm. and 150 mm. embryos the uppermost segments were missing. These were estimated by calculation from the other segments of the same cords, assuming that the same relative increase takes place in these segments as in the other segments of the same cord, when compared with the cord of the 31 mm. embryo. While the obliquity of some of the sections interfered in some respects, these exceptions are carefully noted so that they have not resulted in any great error in the accuracy of the work. The curvature and corresponding obliquity of cross-sections can be determined approximately by the graphic reconstruction in lateral view of the four younger embryos in the paper by Jackson ('09 a).

The exact line of demarcation of gray from white matter was sometimes difficult to determine, due to their intermingling. In the younger embryos in which only the anlagen of the anterior and posterior horns are present, a horizontal line was drawn from the small recess in the boundary zone of the gray matter, which was very thin, to the nearest point of the central canal. This line arbitrarily separated the anterior from the posterior horns. The lateral horn was not present in the younger embryos. In the older stages the lateral horn was included with the anterior, thus dividing the gray matter into a posterior and an antero-lateral horn (figs. 1 to 5).

The white matter was separated into the anterior, posterior, and lateral columns. The lateral border of the anterior column is the line of emergence of the outermost fascicles of the nerve roots. This separates the anterior and lateral columns. The dorso-lateral sulcus at the attachment of the posterior nerve roots separates the posterior and lateral columns. In the 11 mm. specimen the lateral columns showed such an irregularity that they were not separated but measured with the anterior columns. For exact lines of demarcation, see figures 1 to 5.

OBSERVATIONS AND DISCUSSION

A. CORD AS A WHOLE

*1. General form of the prenatal spinal cord (figs. 1 to 12;
tables 2 to 6)*

The spinal cord in the human embryos studied shows various points of resemblance in form to the cord in the adult, as shown in figures 6 to 12. Passing caudad from the brain there are indications, however, that the embryonic spinal cord diminishes gradually in its diameters to about the region of the 3rd cervical segment. This resembles the cord of the child (Stilling), but not of the adult. From here there is in the embryonic cord a slight but constant increase in caliber, which reaches its maximum in the region of the 4th or 5th cervical segment. This enlargement in the cervical region corresponds to the *intumescencia cervicalis*. This is followed by a more gradual decrease in area of cross-section, which usually extends to about the 3rd or 4th thoracic segment. The thoracic segments, from the 3rd to the 8th, have practically the same area of cross-section in each of the embryos examined. This is also true in the child, but to a less extent in the adult. Near the lower end of the thoracic region there are indications of the *intumescencia lumbalis*. The cord in general increases in area of cross-section from the lower thoracic segments to the 5th lumbar segment or thereabout, whence there is a gradual tapering of the cord as it decreases in cross-sectional area to its end in the *filum terminale*. The lower ends of the cords are, therefore, somewhat conical in shape.

In general shape the prenatal cord in cross-section is at first oval, being compressed in its transverse diameter (figs. 1 to 5). Later, however, it becomes compressed in the dorso-ventral diameter and expanded laterally. In the thoracic region, the spinal cord more nearly retains a cylindrical form. In these respects the later fetal cord approaches the postnatal condition. If we compare the increase in cross-sectional area at successive stages with the increase in volume of the spinal cord, we find in general that the volume increases more rapidly. That is, the cord becomes

relatively longer and slenderer during the prenatal life, and this tendency continues in the child to the adult.

*2. Special features in the various embryos: (figs. 1 to 12;
tables 2 to 6)*

In the 11 mm. embryo only the cervical and thoracic segments were measured separately. By referring to table 2 and figure 6, one can readily see that this embryo differs somewhat from the preceding general description. Owing to the obliquity of the section in the cervical and lower thoracic regions (due to the normal curvature of the spinal cord in embryos of this length), not much stress can be laid on the apparent increase in area of cross-section in these regions as shown in the curve. However, from measurements of the transverse diameters (which are not affected by the obliquity) of these sections, which show an increase in the cervical region, the indications are that there is a slight expansion in the region of the cervical enlargement. There is, however, no evident lumbar enlargement. The apparent increase shown in the curve in the lower thoracic region is probably due entirely to the obliquity of the sections. Excepting the slight cervical enlargement, the cord seems to taper from the cephalic end to the caudal extremity. The lower end of the cord is so curved that the segments could not be separated. The shape of the cord in cross-section (figs. 1 a and 1 b) is in general like that of a rectangle with the corners rounded. The outline is not smooth, due probably to the fact that the columns of white matter are as yet not formed.

The spinal cord of the 17 mm. embryo presents (as shown in table 3, figs. 2 a and 2 b, and fig. 7) some of the same features as the cord of the 11 mm. specimen. From the increase in areas of cross-section and in the transverse diameters of the sections, it is evident that there is in this cord a noticeable increase in the region of the cervical enlargement, and a very slight increase in the lower thoracic and upper lumbar segments which is possibly due in part to the beginning of the lumbar enlargement. The actual increases both in the cervical and thoraco-lumbar regions are less

than those apparently shown on the curve, due to the curvature of the cord and the corresponding obliquity of the sections.

In the 31 mm. embryo (table 4, figs. 3 a, 3 b, and 3 c, and fig. 8) the upper three segments are so cut as to contain both medulla and spinal cord. The outline of the cord could be measured separately, however. The cervical enlargement is recognizable also in this cord. This is the youngest embryo in which there is a well-marked lumbar enlargement. This does not agree with Bryce ('08) who states that the cervical and lumbar enlargements are only manifest at the end of the third month. However, Streeter ('11) finds indications of the enlargements at the end of the first month, while Minot ('92) states that they occur at two months (well developed at three months). The cord of the 31 mm. embryo is but slightly larger in area of cross-section (and even smaller in places) than that of the 17 mm. specimen. The difference may be due in part to individual variation and in part to the growth being chiefly along the longitudinal axis about this period.

The upper two cervical segments in the 65 mm. embryo (table 5 and fig. 9) are lacking. They were estimated to complete the data. This was done by assuming that these segments would show the same relative increase as other segments of the same cord, when compared with corresponding segments of the cord in the 31 mm. specimen. They are enclosed in parentheses in the table. A marked variation occurs in this cord. The cervical enlargement appears relatively small. The lumbar enlargement shows a greater area of cross-section than the cervical enlargement. As this relation is not found in any other cord examined, it is due either to more rapid relative growth of the lower portion of the cord at this period or (more probably) to an individual variation. The tapering of the lower extremity of the cord is completely shown here, since all the lower segments were measured separately in this specimen.

In the cord at the middle of the prenatal period (150 mm.: table 6 and fig 10) the upper three cervical segments are missing. These were estimated as above. This cord agrees well with the general description previously given.

3. *Growth of the cord as a whole*

The total volumes of the spinal cords measured are shown in table 1. From these data and volumes of the entire body (which were known) the percentages that the cords bear to the entire body were calculated. They are as follows:

C. R. LENGTH OF EMBRYO	BODY VOLUME	VOLUME OF SPINAL CORD	% OF TOTAL BODY VOLUME
<i>mm.</i>	<i>cc.</i>	<i>cc.</i>	<i>per cent</i>
11	0.976	0.004024	4.130 (4.85)
17	0.3788	0.01194	3.150 (3.43)
31	1.6930	0.02115	1.250 (1.53)
65	(20.00) ¹	0.1505	0.755
150	(200.00) ¹	0.3969	0.198

¹ Estimated

The difference in the percentages obtained by Bonnot and SeEVERS ('06) for the 11 mm., and Jackson ('09 b) for the 17 mm. and 31 mm. specimens, and those by myself is quite marked. Their results are given in parentheses, and are larger in every case. This difference is due chiefly to a difference in technique. In measuring the area of the various sections they took the border on the outer edge of the meninges immediately surrounding the cord, and passed directly over the anterior fissure and posterior sulcus, while I in all cases measured on the surface of the spinal cord proper, leaving out the meninges and following the various breaks in the continuity of the outline. A small difference would be expected due to this difference in technique.

The absolute growth of the prenatal cord is very rapid in the younger embryos as shown by the total volumes of the cords in table 1. This is what is expected, since the neural tube or anlage of the spinal cord develops very early in the embryo. The rate of growth seems, in general, to decrease with the age of the embryo. During the second and third months (11 mm. to 65 mm.), the cord has increased thirty-six times, or 3600 per cent. In the fourth and fifth months (65 mm. to 150 mm.) together the increase is only approximately 160 per cent.

The decrease in the relative growth-rate with age is also shown by the decline in the percentage which the spinal cord forms of the entire body. This agrees with the rate of absolute growth in that as the age increases the percentage becomes relatively smaller. Vierordt ('06) gives 0.18 per cent of the total body weight for the spinal cord in the newborn and 0.06 per cent in the adult. These figures added to my results show that the decrease in growth-rate of the spinal cord continues through prenatal into postnatal life. This agrees with the conclusion reached by Jackson ('09 b).

4. Growth of the various regions

a. Cervical region. The different regions in the spinal cord show in their rates of growth some slight differences when compared with the rate of growth of the entire cord. The cervical region exhibits a slower rate of growth than the whole cord up to the 31 mm. embryo, while from here to the end of the first half of prenatal life (150 mm. embryo) it slightly exceeds the growth rate of the whole cord. The cervical region during this time increases approximately 175 per cent, while the entire cord during the same period increases less than 166 per cent.

The relative amounts by volume which the different regions form of the entire cord are given in table 1. The cervical region in the 11 mm. embryo constitutes 37 per cent of the entire cord. This is probably somewhat too large owing to the obliquity of the cord in this region. There is a slight decrease to about 28 per cent in the mid-fetal cord (150 mm.). In the two-year-old child it is relatively larger, forming 36 per cent of the entire cord. There is a decrease to 31 per cent between this and the adult stage, which seems to correspond to the increase in the thoracic region of the cord.

In area of cross-section, using the 5th cervical segment for comparing the growth of the cervical region, it is observed (tables 3 to 6, also figs. 1 to 12) that the area increases as growth in volume proceeds. However, a comparison of the 17 mm. and 31 mm. embryos shows that the cross-sectional area increases only about 60 per cent while the volume in the same period increases over 100

per cent. This indicates that during this period the growth along the longitudinal axis is greater than in the transverse diameters. The 65 mm. embryo in cross-sectional area shows a small absolute decrease over the 31 mm. embryo in the cervical region. This decrease is probably due to individual variation. During the latter part of the first half of prenatal life the relative growth in area of cross-section continues relatively less than the growth in volume, as compared with the younger stages. By using the areas of the cross-sections of the 5th cervical segment as given by Donaldson and Davis ('03) (taken from Stilling) for a child of two years and a composite adult, an increase of 600 per cent occurs in the child, as compared with the 150 mm. embryo and of only 100 per cent between the child and the adult.

b. Thoracic region. The thoracic region in volume shows an increase of slightly less than 400 per cent between the 11 mm. embryo and the 65 mm. specimen. This is more than the increase in the cervical region during the same time, which is less than 300 per cent. As a result, the thoracic region, which is smaller than the cervical region in the 11 mm. embryo surpasses it in the 17 mm. embryo. From the 65 mm. embryo to the mid-fetal period (150 mm.), the thoracic continues to increase slightly more rapidly than the cervical region. This is also true for the thoracic region when compared with the cord as a whole, as shown in table 1. The thoracic region of the cord continues to increase relatively through postnatal life, forming 50 per cent of the adult cord, while the cervical forms only 31 per cent.

In cross-sectional area the thoracic segments from the 3rd to the 8th are practically constant. The relatively slight increase in cross-sectional area from the 17 mm. to the 65 mm. specimens (tables 3 to 5) is in agreement with the previous statement that during this period the growth is greatest along the longitudinal axis. The cord of the 31 mm. embryo is even slightly smaller in cross-sectional area than that of the 17 mm. embryo in the thoracic region. This absolute decrease is probably due to an individual variation. By using data for the child and adult, taken from Donaldson and Davis ('03) and Stilling ('59) it is shown that in the time which elapses between the mid-fetal

period and the second year of postnatal life the cross-sectional area of the thoracic region increases approximately 1000 per cent, while from here to the adult there is an increase of only about 100 per cent.

c. Lumbo-sacral region. The lumbo-sacral region of the spinal cord shows (table 1) approximately the same rate of growth in volume as the thoracic region and a faster one than the cervical region up to the 17 mm. embryo. The lumbo-sacral region forms 31 per cent of the entire cord in the 11 mm. embryo. It increases relatively until in the 31 mm. specimen, it comprises 38 per cent of the whole cord. The lumbo-sacral region decreases in relative size in later prenatal life until in the child and in the adult it forms only 18 per cent of the entire cord. The sacral region decreases relatively more than the lumbar. After they can be differentiated (31 mm.) the lumbar region is about seventeen times as large as the sacral. In the child, however, the lumbar region is twice as large as the sacral, and in the adult nearly four times as great.

The 4th lumbar segment is used to compare the areas of cross-section in the lumbar region (figs. 1 to 5). In the younger embryos (11 mm. and 17 mm.) this segment could not be measured owing to the curvature of the cord. In the 65 mm. embryo the cross-sectional area of the lumbar region is much larger, relatively and absolutely, than the cervical region, as previously stated. The rate of growth of the lumbar region in area of cross-section is slower than that for the cervical or thoracic regions in the 65 mm. and 150 mm. embryos. This same relative decrease, like that for the volume, continues in this region in the child and adult.

This relative decrease in the lumbar and sacral regions is surprising, for it seems that since the corresponding parts of the body (the pelvis and lower extremities) increase in relative size during this period, we should expect a relative increase in this region of the cord. This decrease is evidently associated with the shortening of the spinal cord within the vertebral canal which begins about the third month and results in the well known retraction of the lower end of the cord.

In comparing the rates of growth in volume and in average cross-sectional area of the spinal cord as a whole, let it be assumed that the volume and the cross-sectional area in the 11 mm. embryo each equals to 1. Then it is noted that in the 17 mm. specimen the volume has increased relatively twice as much as the area of cross-section. In the 31 mm. embryo the increase in length is to the increase in area as 3 is to 2. This relatively greater increase in volume, over the cross-sectional area, is also found in the 65 mm. and 150 mm. embryos, though not so marked in these. This indicates that the growth in length is relatively greater than in area of cross-section, as previously stated.

B. GRAY MATTER

1. General form (tables 2 to 6; figs. 1 to '12) .

The gray matter, which constitutes the cellular part of the spinal cord, in the older embryos studied shows an increase in cross-sectional area in the regions of the enlargement as compared with that found in the thoracic region. In the 11 mm. embryo, (table 2) no enlargement is found. The anterior horns form more than one-half the gray matter in all specimens studied (table 7). In the youngest embryo the anterior horn anlage is approximately three times as large as the posterior horn, which agrees with the statement of His ('86). Later, however, the posterior horns approach the anterior in size, the mid-fetal stage showing relations similar to the adult. The lateral horn is not well marked except in the older embryos (figs. 1 to 5). In all cases when present it is included in the measurements with the anterior horn. The gray matter shows much variation in shape in different segments.

2. Special features in the various regions

In the 11 mm. embryo (table 2 and fig. 6) there seems to be more gray matter (in cross-sectional area) in the cervical than in the thoracic region, but this is at least in part due to the obliquity of the sections. In the 17 mm. embryo (table 3) there

is a slight increase in the area of cross-section in the cervical region (as compared with the thoracic) which probably corresponds to the cervical enlargement. The small increase shown by the lower thoracic and upper lumbar segments is due for the most part to the curvature of the cord. The 31 mm., 65 mm., and 150 mm. embryos all seem to correspond to the general description (tables 4 to 6 and figs. 6 to 12). The 65 mm. embryo shows a larger area of cross-section in the lumbar region than in the cervical. In the others the cervical region is the largest. This is true for the cross-sectional area of the whole cord as well as for the gray matter.

3. Growth as a whole

The total volumes of gray matter, both absolute and relative, are given in table 1. The gray matter comprises about 38 per cent of the entire cord in the 11 mm. embryo. In the 17 mm. embryo it has increased in absolute volume approximately 300 per cent and now comprises about 50 per cent of the entire cord. The increase in absolute volume continues, but relatively slower, to the 65 mm. embryo, where it forms 58 per cent of the total cord. In the 150 mm. embryo (at the mid-fetal period) the relative volume shows a slight decrease (to 53 per cent), while the absolute volume continues to increase. This relative decrease continues into postnatal life, until in the child the gray matter forms only 27 per cent of the whole cord and in the adult only 20 per cent.

As previously stated, the anterior horns are much larger than the posterior in the earlier stages (figs. 1 to 5). The anterior horns can be distinguished in the 11 mm. embryo, although they are far from the characteristic shape assumed later. His ('86) recognized the anlagen of the posterior and anterior horns early in the second month, but states that they do not assume their definite form until later, being very broad at three months. Minot ('92) found them fused at about five months. Streeter ('11) and Bryce ('08), however, state that in the 15 mm. embryo (fifth week) the rudiments of the anterior horns can be seen.

4. *Growth of the various regions (figs. 6 to 12; tables 1 and 7 to 12)*

The cervical region of the cord contains relatively more gray matter by volume than does the cord as a whole. In the 31 mm. embryo the gray matter is 57 per cent of the cervical region, while it is slightly less in the entire cord (55 per cent). This holds true in all the embryos studied, and is seen, by the data on the child and adult, to continue into postnatal life. The gray matter of the cervical region reaches the maximum relative size in the 31 mm. embryo, while in the entire cord the maximum percentage of gray matter is in the 65 mm. specimen. The gray matter in the thoracic region has practically the same rate of growth as the gray matter in the cord as a whole up through the stages examined. The gray matter in the lower regions grows faster than the gray matter in the entire cord up to the 17 mm. embryo, but it grows relatively slower during the rest of the first half of prenatal life.

C. WHITE MATTER

1. *Form (figs. 1 to 5)*

The various columns are indefinitely formed in the 11 mm. embryo and later gradually assume their typical shapes and relations to the gray matter. Even in the mid-fetal stage (150 mm.) the white matter still forms only a comparatively thin layer surrounding the gray matter.

2. *Growth as a whole (table 1)*

The white matter shows a steady increase in the total amount both absolutely and relatively from the youngest embryo examined to the adult, as shown in table 1. In the 11 mm. specimen the white matter forms 13 per cent of the entire cord while in the 150 mm. fetus (mid-fetal period) it constitutes 46 per cent of the whole cord. In the child of two years it forms 73 per cent and in the adult 80 per cent. This is considerably different from the gray matter which, as we have seen, increases relatively

up to the 65 mm. embryo but thereafter decreases relatively into the postnatal life.

The rate of growth for the various columns is somewhat irregular. This may be due to the formation of the different nerve tracts at different periods. Judging from the average cross-sectional areas in table 8, the lateral column in the earlier stages forms more than half of the white matter of the entire cord. Later it decreases in relative size, but is always the largest column, which holds true even in the adult cord. In general the anterior and posterior columns appear relatively small at first, but increase later, approaching the adult proportions in the later fetal days.

3. Growth in the different regions (figs. 1 to 12 and tables 8 to 12)

The white matter in the cervical region of the 11 mm. and 17 mm. embryos, is relatively larger than in the cord as a whole. In the 31 mm. embryo it is about equal and in the 65 mm. and 150 mm. specimens it is less. Over 50 per cent of the total white matter of the 11 mm. embryo is in the cervical region. In the older embryos (from 31 mm.) a greater amount of white matter is found in the thoracic region than in any other. The length of the thoracic region accounts for the larger volume, since the area of cross-section in this region is smaller than in either the cervical or lumbar region. In each of the various regions there is a relative increase of white matter present from the youngest embryo to the adult, as found in the entire cord.

D. EPENDYMA WITH THE CENTRAL CANAL

1. Form (figs. 1 to 12)

The ependyma and central canal are measured together in this study. They undergo some very marked changes during prenatal life. Only the volume and the area in cross-section are considered here. At the cephalic end where it is continuous with the fourth ventricle the canal is usually slightly enlarged. A corresponding enlargement, though somewhat more marked,

is found at the caudal end of the conus medullaris where it forms the sinus rhomboidalis (which is so well marked in birds). After the 17 mm. stage the canal is decidedly narrower in the thoracic region.

2. Growth

The cross-sectional areas of the ependyma and canal are shown in figures 1 to 12, and in tables 1 to 6, while the volumes are given in tables 1 and 9 to 11. The ependyma with the canal in the 11 mm. embryo form 49 per cent of the volume of the entire cord. In the 17 mm. embryo they have decreased in relative size to 24 per cent but show an absolute increase. From this stage they show a decrease both in relative and in actual size until in the mid-fetal period (150 mm.) they form only 0.59 per cent of the whole cord. This decrease corresponds to the closure of the dorsal part of the central canal as described by His ('86). The ependyma and canal are too small to be shown in the curves after the 65 mm. stage. It appears that the ependyma and canal reach their maximum (absolute as well as relative) size during the second month, decreasing steadily thereafter. This agrees with Streeter ('11) who finds them relatively and absolutely largest in the 15 mm. embryo. Minot ('92), however, says the canal remains stationary from the third to fifth months of prenatal life.

It seems that from the 17 mm. to the 65 mm. stage the gray and white matter both grow chiefly at the expense of the canal and ependyma; thereafter the white matter continues to increase, while the gray matter decreases in relative volume (percentage of the entire cord).

SUMMARY

Some of the more important observations and conclusions concerning the growth of the spinal cord, may be summarized as follows:

1. In the 11 mm. embryo indications of the cervical enlargement appear. In the 31 mm. embryo the lumbar enlargement is first definitely shown, though it may also be present at 17 mm.

The 11 mm. cord in general tapers from the cervical end to the caudal extremity. In the 65 mm. and the 150 mm. stages the cervical and lumbar enlargements appear very prominent.

2. The percentage which the spinal cord forms of the entire body declines rapidly during the second and third months of prenatal life and later more slowly, as shown by Jackson. The actual rate of absolute growth of the cord is much more rapid during the early prenatal months than during the later periods.

3. The various regions of the cord form different percentages of the whole cord at different ages. The cervical region forms approximately 37 per cent of the whole cord in the 11 mm. embryo and decreases to 28 per cent in the mid-fetal stage (150 mm.). In the child and adult it forms 36 per cent and 31 per cent of the whole, respectively. In the thoracic region there is a gradual increase from 32 per cent in the 11 mm. embryo to 41 per cent in the (150 mm.) mid-fetal stage, to 45 per cent in the child, and 50 per cent in the adult. The lumbo-sacral region of the cord increases relatively from 31 per cent in the 11 mm. embryo to a maximum of 38 per cent at 31 mm. This is followed by a gradual decrease to 31 per cent in the mid-fetal stage and to 18 per cent in both child and adult. This decrease in relative size which occurs from the second month of prenatal life and extends into the postnatal period, is associated with the shortening of the cord in the vertebral canal. It is very remarkable when compared with the relative increase at the same time in the corresponding portions of the body (pelvis and lower extremities). This decrease is most marked in the sacral region of the cord. The thoracic region appears to grow at the expense of the cervical region up to about the second month of prenatal life, and thereafter at the expense of the lumbo-sacral region, continuing up to the adult cord.

4. The gray matter constitutes about 38 per cent of the whole cord in the 11 mm. embryo increasing relatively to about 58 per cent in the 65 mm. specimen. Thereafter it decreases until in the child it forms 27 per cent and in the adult less than 20 per cent of the whole cord. The relative amount of gray matter in the cervical region from the earliest stages, and in the lumbo-

sacral region from the 31 mm. stage, is slightly greater than that in the thoracic region. The anterior horn is about three times as large as the posterior horn in the youngest embryo (11 mm.). This difference in size becomes less in the later stages where the ratio approaches that found in the adult cord.

5. The white matter has a rate of growth different from that of the gray matter. It increases steadily from 13 per cent in the 11 mm. stage to 46 per cent of the whole cord in the mid-fetal period (150 mm.). In the child it forms 73 per cent and in the adult 80 per cent, showing that the steady increase continues into postnatal life. In the white matter, as also in the gray matter, the relative increase in the different regions is about the same as the increase in the cord as a whole. The different columns of white matter present irregularities in growth which may be due to the successive formation of various tracts at different ages. The lateral column appears always the largest, however, especially in the earlier stages.

6. The ependyma with the canal show some interesting growth relations. In the 11 mm. embryo they form nearly 50 per cent of the entire cord. This is followed by a rapid relative decrease until by middle of the fetal period (150 mm.) they form only 0.59 per cent of the whole. This marked relative decrease is accompanied by a decrease in the absolute size from the 17 mm. stage onward. With the exception of a slight dilation at the extremities, the canal is fairly uniform in caliber in the 11 mm. and 17 mm. stages, but from the 31 mm. stage onward it is more constricted in the thoracic region. The white and gray matter both grow at the expense of the ependyma and canal until about the third month, when the gray matter begins to decrease in relative amount while the white matter continues to increase.

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TABLE 1

Showing total volumes of spinal cords of various ages; the absolute and relative amounts of gray matter, of white matter, of ependyma with the canal, and of the different regions of the cord

Embryo No.....	60	58	57	55	54	Child	Adult
						(Stillling)	
Length in mm.....	11	17	31	65	150		
Age in days (estimated).....	33	41	56	81	140	2 years	
Total volume of cord (cc.).....	0.00402	0.01194	0.02115	0.1505	0.3969	7.424	27.327
Volume of gray matter (cc.)...	0.00152	0.00594	0.01161	0.0878	0.2115	1.981	5.353
Volume of white matter (cc.)...	0.00053	0.00308	0.00686	0.0603	0.1831	5.443	21.974
Volume of ependyma and canal.....	0.00197	0.00292	0.00268	0.0024	0.0023		
Gray matter (%).....	37.93	49.76	54.90	58.35	53.29	26.68	19.58
White matter (%).....	13.18	25.79	32.43	40.05	46.12	73.32	80.42
Ependyma and canal (%).....	48.89	24.45	12.67	1.60	0.59		
Volume of cervical region (cc.)...	0.00148	0.00376	0.00572	0.0419	0.1098	2.697	8.557
Volume of thoracic region (cc.)...	0.00129	0.00410	0.00730	0.0589	0.1640	3.359	13.662
Volume of lumbar region (cc.).....			0.00510	0.0315	0.0771	0.915	4.012
Volume of sacral region (cc.).....	0.00125	0.00408					
Cervical region (%).....	36.75	31.52	27.09	27.82	27.66	36.33	31.33
Thoracic region (%).....	32.19	34.32	34.94	39.12	41.82	45.34	50.00
Lumbar region (%).....			24.11	20.93	19.43	12.32	14.68
Sacral region (%).....	31.06	34.16	13.86	12.13	11.59	6.01	3.99

TABLE 2

Areas of cross-sections of the spinal cord in an 11 mm. human embryo, showing the absolute and relative amounts of gray matter, of white matter, and of ependyma with canal

SEGMENT	AREA OF CROSS- SECTION	AREA OF GRAY MATTER	AREA OF WHITE MATTER	AREA OF EPENDYMA AND CANAL	% OF GRAY MATTER	% OF WHITE MATTER	% OF EPENDYMA AND CANAL
	<i>sq. mm.</i>	<i>sq. mm.</i>	<i>sq. mm.</i>	<i>sq. mm.</i>			
Cervical I	0.600	0.244	0.120	0.236	40.67	20.00	39.33
Cervical II	0.560	0.232	0.112	0.216	41.43	20.00	38.57
Cervical III	0.540	0.224	0.112	0.204	41.48	20.74	37.78
Cervical IV	0.528	0.220	0.108	0.200	41.67	20.45	37.88
Cervical V	0.544	0.204	0.104	0.236	37.50	19.12	43.38
Cervical VI	0.532	0.208	0.084	0.240	39.10	15.79	45.11
Cervical VII	0.508	0.224	0.072	0.212	44.10	14.17	41.73
Cervical VIII	0.464	0.168	0.068	0.228	36.21	14.65	49.14
Thoracic I	0.436	0.160	0.064	0.212	36.70	14.86	48.62
Thoracic II	0.416	0.148	0.060	0.208	35.58	14.42	50.00
Thoracic III	0.392	0.136	0.056	0.200	34.69	14.29	51.02
Thoracic IV	0.384	0.148	0.044	0.192	38.54	11.46	50.00
Thoracic V	0.364	0.144	0.040	0.180	39.56	10.99	49.45
Thoracic VI	0.380	0.132	0.056	0.192	34.73	14.74	50.53
Thoracic VII	0.384	0.148	0.052	0.184	38.54	13.54	47.92
Thoracic VIII	0.376	0.144	0.044	0.188	38.30	11.70	50.00
Thoracic IX	0.372	0.136	0.052	0.184	36.56	13.98	49.46
Thoracic X	0.344	0.136	0.040	0.168	39.53	11.63	48.84
Thoracic XI	0.364	0.132	0.044	0.188	36.26	12.00	51.65
Thoracic XII	0.396	0.144	0.044	0.208	36.36	11.11	52.53

TABLE 3

Areas of cross-sections of the spinal cord in a 17 mm. human embryo, showing the absolute and relative amounts of gray matter, of white matter, and of ependyma with canal

SEGMENT	AREA OF CROSS- SECTION	AREA OF GRAY MATTER	AREA OF WHITE MATTER	AREA OF EPENDYMA AND CANAL	% OF GRAY MATTER	% OF WHITE MATTER	% OF EPENDYMA AND CANAL
	<i>sq. mm.</i>	<i>sq. mm.</i>	<i>sq. mm.</i>	<i>sq. mm.</i>			
Cervical I	1.300	0.644	0.376	0.280	49.54	28.92	21.54
Cervical II	1.188	0.596	0.356	0.236	50.17	29.97	19.86
Cervical III	1.152	0.576	0.348	0.228	50.00	30.21	19.79
Cervical IV	1.180	0.576	0.372	0.232	48.81	31.53	19.66
Cervical V	1.264	0.652	0.372	0.240	51.58	29.43	18.99
Cervical VI	1.256	0.636	0.376	0.244	50.64	29.93	19.43
Cervical VII	1.232	0.636	0.340	0.256	51.62	27.60	20.78
Cervical VIII	1.128	0.560	0.340	0.228	49.64	30.14	20.22
Thoracic I	1.036	0.524	0.280	0.232	50.58	27.03	22.39
Thoracic II	0.932	0.444	0.272	0.216	47.64	29.18	23.18
Thoracic III	0.924	0.448	0.256	0.220	48.49	27.70	23.81
Thoracic IV	0.880	0.424	0.248	0.208	48.18	28.18	23.64
Thoracic V	0.920	0.464	0.240	0.216	50.44	26.08	23.48
Thoracic VI	0.932	0.484	0.244	0.204	51.93	26.18	21.89
Thoracic VII	0.908	0.460	0.232	0.216	50.66	25.55	23.79
Thoracic VIII	0.900	0.444	0.232	0.224	49.33	25.78	24.89
Thoracic IX	0.916	0.456	0.232	0.228	49.78	25.33	24.89
Thoracic X	0.896	0.428	0.232	0.236	47.77	25.89	26.34
Thoracic XI	0.872	0.420	0.220	0.232	48.16	25.23	26.61
Thoracic XII	0.956	0.448	0.248	0.260	46.86	25.94	27.20
Lumbar I	1.044	0.492	0.268	0.284	47.13	25.67	27.20
Lumbar II	1.076	0.516	0.276	0.284	47.96	25.65	26.39
Lumbar III	1.128	0.540	0.292	0.296	47.87	25.89	26.24

TABLE 4

Areas of cross-sections of the spinal cord in a 31 mm. human embryo, showing the absolute and relative amounts of gray matter, of white matter, and of ependyma with canal

SEGMENT		AREA OF CROSS- SECTION	AREA OF GRAY MATTER	AREA OF WHITE MATTER	AREA OF EPENDYMA AND CANAL	% OF GRAY MATTER	% OF WHITE MATTER	% OF EPENDYMA AND CANAL
		sq. mm.	sq. mm.	sq. mm.	sq. mm.			
Cervical	I	2.166	1.175	0.701	0.290	54.25	32.36	13.39
Cervical	II	2.142	1.182	0.688	0.272	55.18	32.12	12.70
Cervical	III	2.080	1.160	0.670	0.250	55.77	32.21	12.02
Cervical	IV	2.112	1.204	0.672	0.236	57.01	31.82	11.17
Cervical	V	1.984	1.120	0.668	0.196	56.45	33.67	9.88
Cervical	VI	1.780	0.992	0.596	0.192	55.73	33.38	10.89
Cervical	VII	1.496	0.808	0.532	0.156	54.01	35.56	10.43
Cervical	VIII	1.268	0.652	0.488	0.128	51.42	38.49	10.09
Thoracic	I	1.100	0.564	0.424	0.112	51.27	38.55	10.18
Thoracic	II	0.904	0.460	0.352	0.092	54.88	38.94	10.18
Thoracic	III	0.852	0.416	0.348	0.088	48.83	40.84	10.33
Thoracic	IV	0.816	0.400	0.328	0.088	49.02	40.20	10.78
Thoracic	V	0.764	0.384	0.296	0.084	50.26	38.74	11.00
Thoracic	VI	0.812	0.404	0.320	0.088	49.75	39.41	10.84
Thoracic	VII	0.808	0.400	0.324	0.084	49.50	40.10	10.40
Thoracic	VIII	0.848	0.436	0.308	0.104	51.42	36.32	12.26
Thoracic	IX	0.860	0.436	0.312	0.112	50.70	36.28	13.02
Thoracic	X	0.944	0.492	0.320	0.132	52.12	33.90	13.98
Thoracic	XI	0.992	0.536	0.332	0.124	54.03	33.47	12.50
Thoracic	XII	1.206	0.708	0.368	0.140	58.22	30.26	11.52
Lumbar	I	1.248	0.672	0.412	0.164	53.85	33.01	13.14
Lumbar	II	1.428	0.768	0.432	0.228	53.84	33.02	13.14
Lumbar	III	1.488	0.832	0.436	0.220	53.78	30.25	15.97
Lumbar	IV	1.540	0.848	0.476	0.216	55.91	29.30	14.79
Lumbar	V	1.528	0.832	0.460	0.236	55.45	30.11	15.44
Sacral	I	1.392	0.764	0.408	0.220	54.89	29.31	15.80
Sacral	II	1.372	0.748	0.400	0.224	54.52	29.15	16.33

TABLE 5

Areas of cross-sections of the spinal cord in a 65 mm. human embryo, showing absolute and relative amounts of gray matter, of white matter, and of ependyma with canal

SEGMENT	AREA OF CROSS- SECTION	AREA OF GRAY MATTER	AREA OF WHITE MATTER	AREA OF EPENDYMA AND CANAL	% OF GRAY MATTER	% OF WHITE MATTER	% OF EPENDYMA AND CANAL
	<i>sq. mm.</i>	<i>sq. mm.</i>	<i>sq. mm.</i>	<i>sq. mm.</i>			
Cervical I	(1.936)	(1.016)	(0.880)	(0.040)	(52.48)	(45.45)	(2.07)
Cervical II	(1.832)	(1.000)	(0.796)	(0.036)	(54.59)	(43.45)	(1.96)
Cervical III	1.772	0.976	0.764	0.032	55.08	43.12	1.80
Cervical IV	1.808	0.992	0.784	0.032	54.87	43.36	1.77
Cervical V	1.896	1.028	0.832	0.036	54.22	43.88	1.90
Cervical VI	1.756	1.008	0.716	0.032	57.40	40.78	1.82
Cervical VII	1.592	0.904	0.664	0.024	56.78	41.71	1.51
Cervical VIII	1.376	0.756	0.592	0.028	54.94	43.02	2.04
Thoracic I	1.120	0.612	0.484	0.024	54.64	43.21	2.15
Thoracic II	1.020	0.588	0.408	0.024	55.65	40.00	2.35
Thoracic III	1.044	0.604	0.420	0.020	57.85	40.23	1.92
Thoracic IV	0.960	0.556	0.384	0.020	57.92	40.00	2.08
Thoracic V	0.988	0.548	0.420	0.020	55.46	42.51	2.03
Thoracic VI	0.980	0.564	0.392	0.024	57.55	40.00	2.45
Thoracic VII	1.040	0.576	0.440	0.024	55.38	42.31	2.31
Thoracic VIII	1.024	0.572	0.424	0.028	55.86	41.41	2.73
Thoracic IX	1.096	0.612	0.456	0.028	55.84	41.61	2.55
Thoracic X	1.208	0.680	0.500	0.028	56.29	41.39	2.32
Thoracic XI	1.244	0.724	0.492	0.028	58.20	39.55	2.25
Thoracic XII	1.508	0.860	0.616	0.032	57.03	40.85	2.12
Lumbar I	1.820	1.036	0.748	0.036	56.92	41.10	1.98
Lumbar II	2.184	1.224	0.912	0.048	56.04	41.76	2.20
Lumbar III	2.416	1.404	0.972	0.040	58.11	40.23	1.66
Lumbar IV	2.560	1.516	1.000	0.044	59.22	39.06	1.72
Lumbar V	2.412	1.448	0.920	0.044	60.03	38.14	1.83
Sacral I	2.344	1.420	0.872	0.052	60.58	37.20	2.22
Sacral II	1.828	1.120	0.660	0.048	61.27	36.10	2.63
Sacral III	1.416	0.892	0.492	0.032	62.99	34.75	2.26
Sacral IV	1.032	0.616	0.384	0.032	59.69	37.21	3.10
Sacral V	0.560	0.288	0.252	0.020	51.43	45.00	3.57
Coccygeal.....	0.360	0.216	0.124	0.020	60.00	34.45	5.55
Conus med.....	0.244	0.100	0.132	0.012	40.98	54.10	4.92
Filum term.....	0.168	0.016	0.128	0.024	9.52	76.19	14.29

TABLE 6

Areas of cross-sections of the spinal cord in a 150 mm. human embryo, showing absolute and relative amounts of gray matter, of white matter, and of ependyma with canal

SEGMENT	AREA OF CROSS- SECTION	AREA OF GRAY MATTER	AREA OF WHITE MATTER	AREA OF EPENDYMA AND CANAL	% OF GRAY MATTER	% OF WHITE MATTER	% OF EPENDYMA AND CANAL
	sq. mm.	sq. mm.	sq. mm.	sq. mm.			
Cervical I	(7.728)	(4.452)	(3.220)	(0.056)	(57.61)	(41.67)	(0.72)
Cervical II	(7.460)	(4.160)	(3.248)	(0.052)	(55.82)	(43.54)	(0.64)
Cervical III	(7.360)	(4.032)	(3.280)	(0.048)	(54.78)	(44.57)	(0.65)
Cervical IV	7.386	4.040	3.304	0.044	54.68	44.72	0.60
Cervical V	7.560	4.280	3.232	0.048	56.61	42.75	0.64
Cervical VI	7.748	4.476	3.224	0.048	57.77	41.61	0.62
Cervical VII	7.164	4.036	3.080	0.048	56.34	42.99	0.67
Cervical VIII	5.256	2.676	2.540	0.040	50.91	48.33	0.76
Thoracic I	3.640	1.816	1.800	0.024	49.89	49.45	0.66
Thoracic II	2.812	1.496	1.296	0.020	53.20	46.09	0.71
Thoracic III	2.412	1.256	1.140	0.016	52.07	47.26	0.67
Thoracic IV	2.524	1.308	1.204	0.012	51.82	47.70	0.48
Thoracic V	2.404	1.236	1.156	0.012	51.41	48.09	0.50
Thoracic VI	2.500	1.260	1.288	0.012	50.40	49.12	0.48
Thoracic VII	2.464	1.252	1.196	0.016	50.81	48.51	0.65
Thoracic VIII	2.420	1.216	1.188	0.016	50.25	49.09	0.66
Thoracic IX	2.652	1.384	1.252	0.016	52.19	47.21	0.60
Thoracic X	2.832	1.464	1.352	0.016	51.69	47.74	0.57
Thoracic XI	3.132	1.632	1.480	0.020	52.11	47.25	0.64
Thoracic XII	3.636	1.812	1.804	0.020	49.84	49.62	0.54
Lumbar I	4.228	2.264	1.944	0.020	53.55	45.98	0.47
Lumbar II	5.268	3.028	2.220	0.020	57.48	42.14	0.38
Lumbar III	5.796	3.328	2.444	0.024	57.42	42.17	0.41
Lumbar IV	6.140	3.584	2.536	0.020	58.37	41.30	0.33
Lumbar V	6.080	3.620	2.440	0.020	59.54	40.13	0.33
Sacral I	5.792	3.396	2.372	0.024	58.63	40.95	0.42
Sacral II	4.948	2.644	2.284	0.020	53.44	46.16	0.40
Sacral III	4.204	2.272	1.196	0.016	54.04	45.58	0.38
Sacral IV	3.540	2.040	1.480	0.020	57.63	41.81	0.56
Sacral V	3.104	1.772	1.304	0.028	57.09	42.01	0.90
Coccygeal.....	2.296	1.220	1.048	0.028	53.14	45.64	1.22
Conus med.....	1.644	0.884	0.732	0.028	53.77	44.53	1.70
Filum term.....	0.424	0.032	0.280	0.112	7.55	66.04	26.41

TABLE 7

Showing the average cross-sectional area of gray matter in the various regions, and the relative amounts of the anterior and posterior horns

REGION		EMBRYO					CHILD	ADULT
		11 mm.	17 mm.	31 mm.	65 mm.	150 mm.	(Stillling)	
Cervical V, VI, VII, VIII.....	Area of gray matter (sq. mm.).....	0.201	0.621	0.893	0.924	3.867	15.91	17.89
	(%) Ant. horn.....	74.63	69.40	58.45	62.45	54.69		58.80
	(%) Post. horn.....	25.37	30.60	41.55	37.55	45.31		41.20
	Area of gray matter (sq. mm.).....	0.142	0.454	0.460	0.625	1.382	6.45	5.36
Thoracic.....	(%) Ant. horn.....	79.58	72.03	58.69	64.48	53.62		49.16
	(%) Post. horn.....	20.42	27.97	41.31	35.52	46.38		50.84
	Area of gray matter (sq. mm.).....		0.515	0.790	1.326	3.165	14.55	14.41
Lumbar.....	(%) Ant. horn.....		73.59	56.58	57.16	55.04		52.80
	(%) Post. horn.....		26.41	43.42	42.84	44.96		47.20

TABLE 8

Showing the average cross-sectional area of white matter in the various regions, and the relative amounts of the anterior, lateral, and posterior columns

REGION		EMBRYO					CHILD	ADULT
		11 mm.	17 mm.	31 mm.	65 mm.	150 mm.	(Stillling)	
Cervical V, VI, VII, VIII.....	Area of white matter (sq. mm.).....	0.082	0.357	0.571	0.701	3.019	42.24	37.64
	(%) Post. column.....	36.59	20.45	35.20	28.10	25.55		33.00
	(%) Lat. column.....	63.41	59.38	51.14	48.08	41.75		36.40
	(%) Ant. column.....		20.17	13.66	23.82	32.70		30.60
	Area of white matter (sq. mm.).....	0.050	0.245	0.336	0.453	1.387	23.47	24.22
Thoracic.....	(%) Post. column.....	38.00	20.41	35.71	26.27	32.30		27.60
	(%) Lat. column.....	62.00	60.00	51.49	46.14	45.93		54.05
	(%) ant. column.....		10.59	12.80	27.59	21.77		18.35
	Area of white matter (sq. mm.).....		0.279	0.444	0.910	2.321	21.88	20.80
Lumbar.....	(%) Post. column.....		20.43	26.80	31.65	34.38		30.20
	(%) Lat. column.....		56.99	53.40	47.47	40.84		39.80
	(%) Ant. column.....		22.58	19.80	20.88	24.78		31.00

TABLE 9

Absolute and relative volumes of white matter, of gray matter, and of ependyma with the canal in various regions of the cord in the 11 mm. and 17 mm. embryos

REGION		11 MM. EMBRYO			17 MM. EMBRYO		
		Volume	% of region	% of total	Volume	% of region	% of total
Cervical.....	cc.						
	White matter.....	0.000269	18.18	50.69	0.001094	29.07	35.54
	Gray matter.....	0.000643	43.48	42.14	0.001919	51.00	32.31
	Canal and ependyma.....	0.000567	38.34	28.81	0.000750	19.93	25.69
Thoracic.....	White matter.....	0.000166	12.82	31.38	0.001073	26.20	34.86
	Gray matter.....	0.000485	37.45	31.77	0.002005	48.95	33.76
	Canal and ependyma.....	0.000644	49.73	32.73	0.001018	24.85	34.88
	White matter.....	0.000095	7.60	17.92	0.000911	22.35	29.60
Lumbo-Sacral ..	Gray matter.....	0.000398	31.84	26.09	0.002015	49.42	33.93
	Canal and ependyma.....	0.000757	60.56	38.46	0.001151	28.23	39.43

TABLE 10

Absolute and relative volumes of white matter, of gray matter, and of ependyma with the canal in various regions of the cord in the 31 mm. and 65 mm. embryos

REGION		31 MM. EMBRYO			65 MM. EMBRYO		
		Volume	% of region	% of total	Volume	% of region	% of total
		cc.			cc.		
Cervical.....	White matter.....	0.00187	32.64	27.26	0.01625	38.80	26.97
	Gray matter.....	0.00326	56.89	28.08	0.02480	59.22	28.49
	Canal and ependyma	0.00060	10.47	22.39	0.00083	1.98	25.70
Thoracic.....	White matter.....	0.00276	37.35	40.23	0.02644	44.90	43.88
	Gray matter.....	0.00427	55.07	35.06	0.03181	54.02	36.55
	Canal and ependyma	0.00056	7.58	20.89	0.00064	1.08	19.81
Lumbar.....	White matter.....	0.00157	30.78	22.89	0.01182	37.52	19.61
	Gray matter.....	0.00284	55.64	24.46	0.01860	59.05	21.37
	Canal and ependyma	0.00069	13.58	25.75	0.00108	3.43	33.44
Sacral.....	White matter.....	0.00066	22.52	9.62	0.00575	31.49	9.54
	Gray matter.....	0.00144	49.15	12.40	0.01183	64.79	13.59
	Canal and ependyma	0.00083	28.33	30.97	0.00068	3.72	21.05

TABLE 11

Absolute and relative volumes of white matter, of gray matter, and of ependyma with the canal in various regions of the cord in the 150 mm. embryo.

REGION		VOLUME	% OF REGION	% OF TOTAL
		cc.		
Cervical...	White matter.....	0.04848	44.16	26.47
	Gray matter.....	0.06059	55.19	28.65
	Canal and ependyma.....	0.00071	0.65	30.60
Thoracic...	White matter.....	0.08087	49.31	44.16
	Gray matter.....	0.08213	50.09	38.82
	Canal and ependyma.....	0.00100	0.60	43.10
Lumbar...	White matter.....	0.03336	43.25	18.22
	Gray matter.....	0.04345	56.33	20.57
	Canal and ependyma.....	0.00032	0.42	13.80
Sacral.....	White matter.....	0.02043	44.41	11.15
	Gray matter.....	0.02528	54.96	11.96
	Canal and ependyma.....	0.00029	0.63	12.50

TABLE 12

Relative volumes of white matter and of gray matter in the various regions of the spinal cord in a child of two years (Stilling) and in a composite adult (Donaldson and Davis).

REGION		CHILD		ADULT	
		% of region	% of total	% of region	% of total
Cervical...	White matter.....	75.60	37.46	80.35	31.27
	Gray matter.....	24.97	33.23	19.65	31.41
Thoracic...	White matter.....	78.26	48.29	85.58	53.20
	Gray matter.....	21.74	36.87	14.42	36.89
Lumbar...	White matter.....	63.04	10.59	70.49	12.99
	Gray matter.....	36.94	17.06	29.53	22.14
Sacral.....	White matter.....	43.90	3.66	53.28	2.54
	Gray matter.....	56.10	12.84	46.72	9.56

EXPLANATION OF FIGURES

Figures 1 to 5 represent outline drawings of actual cross-sections of different regions in the various spinal cords studied. $\times 12$. Where the sections drawn did not show any nerve roots, the lines of separation for the various columns of white matter were approximated. *C*, central canal; *E*, ependyma; *P*, posterior horns of gray matter; *A*, anterior horns of gray matter; *l*, lateral columns of white matter; *p*, posterior columns of white matter; *a*, anterior columns of white matter.

Fig. 1 a 5th cervical segment, 11 mm. embryo.

Fig. 1 b 5th thoracic segment, 11 mm. embryo.

Fig. 2 a 5th cervical segment, 17 mm. embryo.

Fig. 2 b 5th thoracic segment, 17 mm. embryo.

Fig. 3 a 5th cervical segment, 31 mm. embryo.

Fig. 3 b 5th thoracic segment, 31 mm. embryo.

Fig. 3 c 4th lumbar segment, 31 mm. embryo.

Fig. 4 a 5th cervical segment, 65 mm. embryo.

Fig. 4 b 6th thoracic segment, 65 mm. embryo.

Fig. 4 c 4th lumbar segment, 65 mm. embryo.

Fig. 5 a 5th cervical segment, 150 mm. embryo.

Fig. 5 b 6th thoracic segment, 150 mm. embryo.

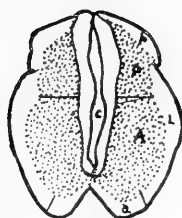
Fig. 5 c 4th lumbar segment, 150 mm. embryo.



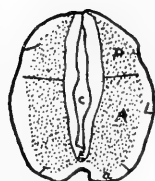
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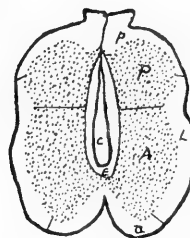
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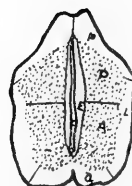
2 a



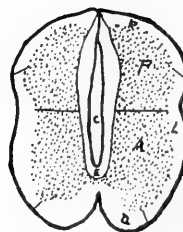
2 b



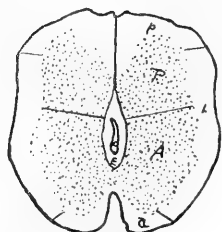
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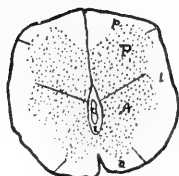
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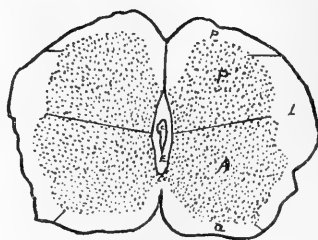
3 c



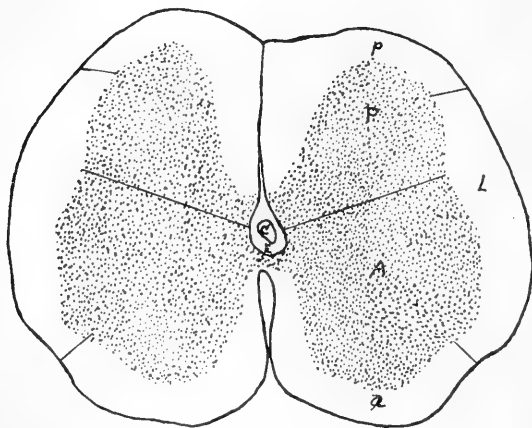
4 a



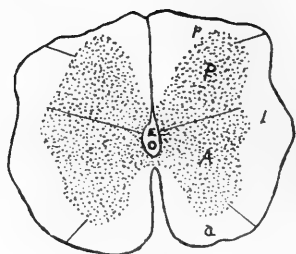
4 b



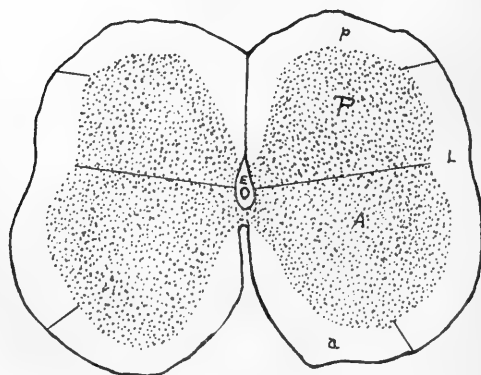
4 c



5 a



5 b



5 c

EXPLANATION OF FIGURES

Figures 6 to 12 represent by curves the cross-sectional areas in each segment of several embryonic and adult human spinal cords, as well as the corresponding areas of gray and white matter (also the ependyma with the canal in figures 6 to 9). The curves are so plotted that the areas enclosed between the base-lines and curves represent the total volumes of the cords and of their component parts, respectively. The figures are so drawn that the areas representing the total volumes of the cords are approximately the same. The lengths of the segments are represented on the abscissa and so calculated that the total lengths of the various cords are represented by the same length of abscissa.

In any given figure, the changes in the height of the curves therefore represent changes in the caliber of the cord as a whole (or in the relative amounts of its component parts) at different levels. A comparison of the different figures shows for the various stages the changes in the form of the cord as a whole, and in the relative amounts of the component parts. The following points must be held in mind to avoid error in comparing the various curves:

1. Curves of figures 6, 7 and 8 are incomplete at the lower end.
2. Curves of figures 9 and 10 are estimated at the upper end (dotted lines) as explained in the text.
3. The apparent increase at the upper end (all of the cervical region) of figure 6 is mostly due to the obliquity of the sections corresponding to the curvature of the spinal cord. This also applies to the lower six thoracic segments of figure 6, to the lumbar segments of figure 7, and to the upper four cervical, to some extent, in figure 8. In figure 9, all the cervical segments are thus slightly enlarged, although not enough to require dotted lines.

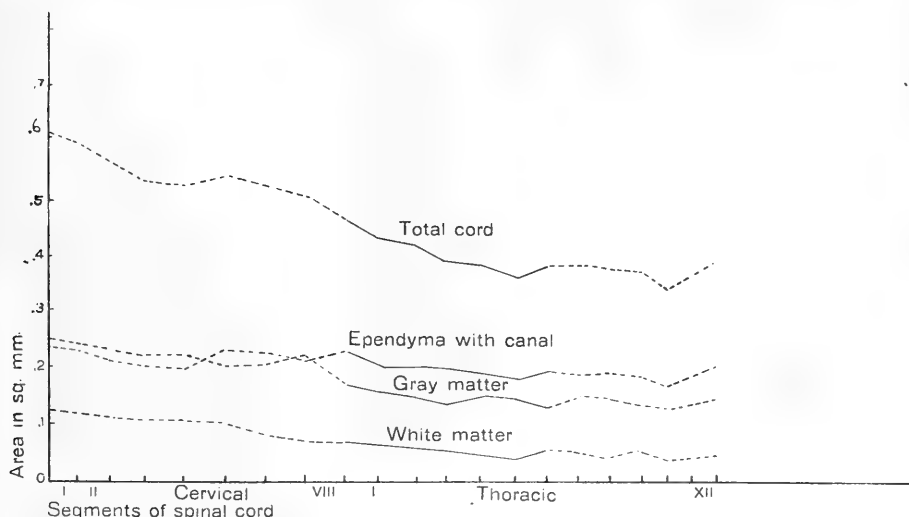


Fig. 6 Spinal cord of human embryo of 11 mm.

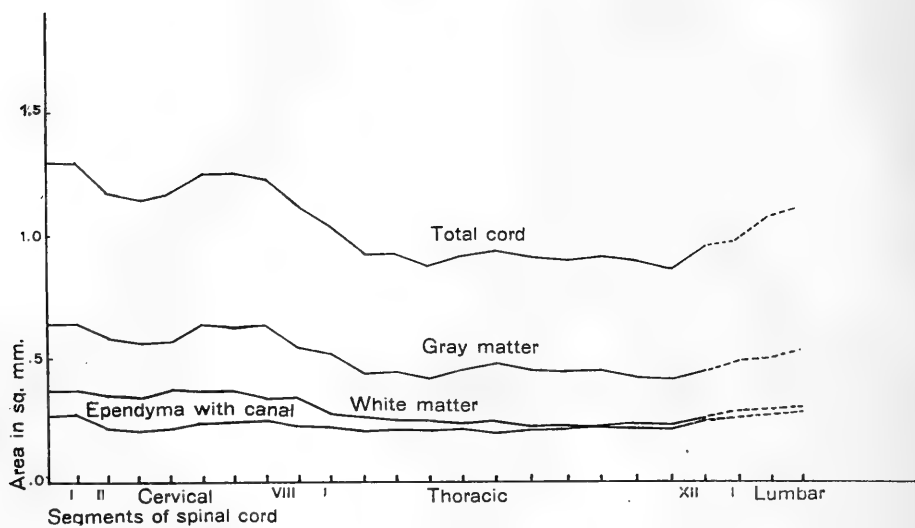


Fig. 7 Spinal cord of human embryo of 17 mm.

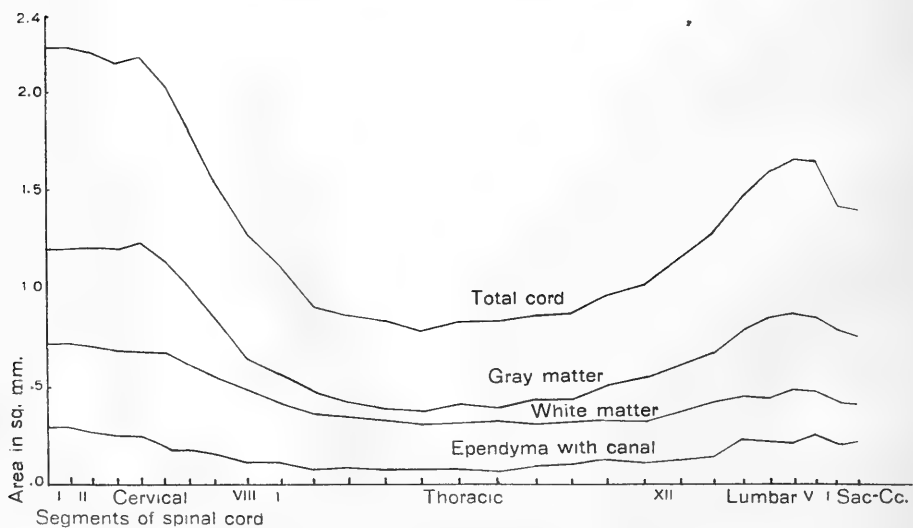


Fig. 8 Spinal cord of human embryo of 31 mm.

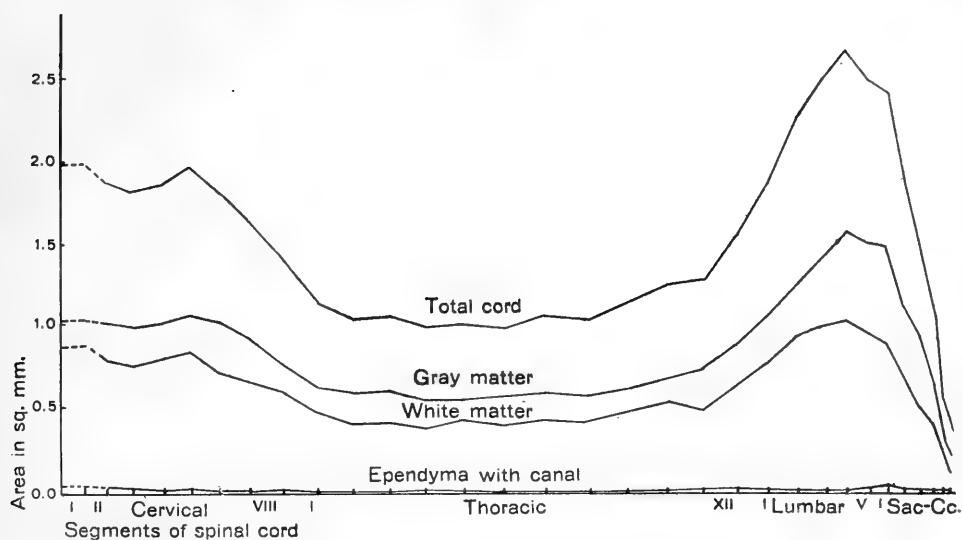


Fig. 9 Spinal cord of human embryo of 65 mm.

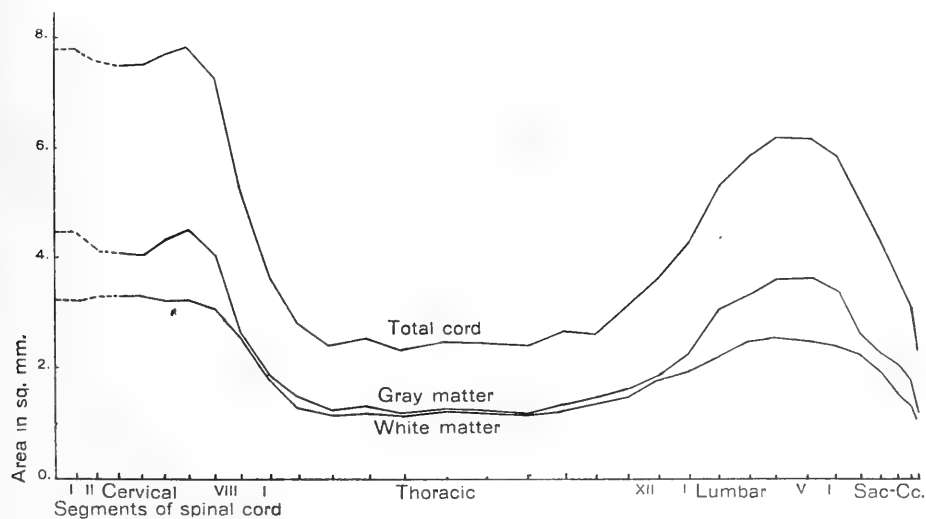


Fig. 10 Spinal cord of human embryo of 150 mm.

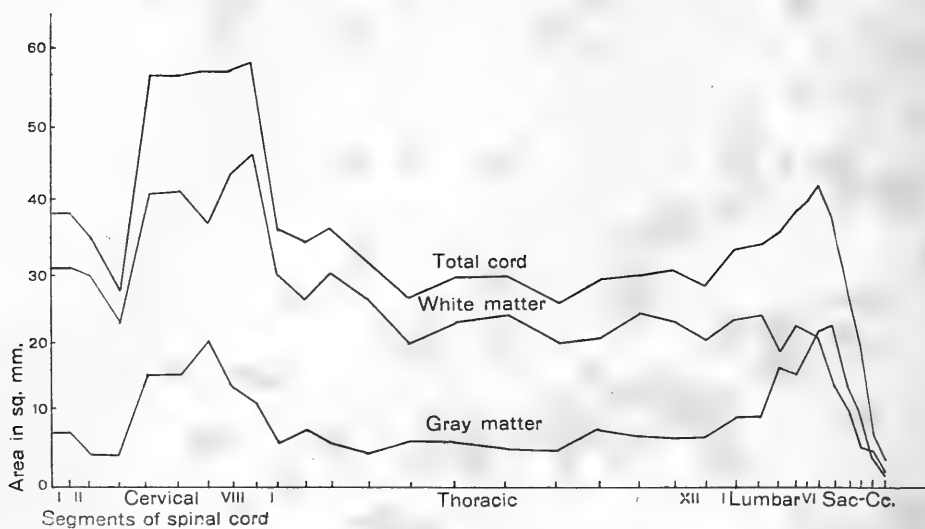


Fig. 11 Spinal cord of a two-year-old child; data taken from Stilling's observations.

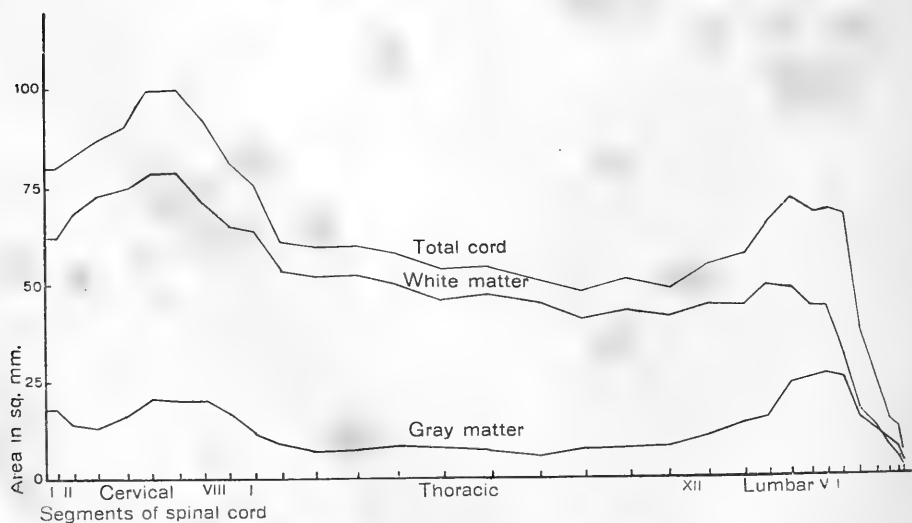


Fig. 12 Spinal cord of a composite adult; data from Donaldson and Davis, which were calculated from the data of four adult cords given by Stilling.

THE DEVELOPMENT OF THE CRANIAL SYMPATHETIC GANGLIA IN THE PIG

ALBERT KUNTZ

From the Laboratories of Animal Biology of the State University of Iowa

FIFTEEN FIGURES

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INTRODUCTION

A review of the literature bearing on the development of the sympathetic nervous system in the vertebrate series shows that in the general investigations of the sympathetic system the cranial sympathetic ganglia, viz., the ciliary, the sphenopalatine, the otic and the submaxillary ganglia, have received but little attention. The development of the ciliary ganglion has been studied by not a few investigators in connection with the eye-muscle nerves. On the development of the remaining cranial sympathetic ganglia, only fragmentary and incomplete observations have been recorded.

A systematic review of the literature bearing on the development of the cranial sympathetic ganglia will not be attempted

in this paper. Carpenter ('06) has given us a more or less exhaustive review of the literature bearing on the development of the ciliary ganglion in the entire vertebrate series in his paper on the development of the oculomotor nerve, the ciliary ganglion, and the abducent nerve in the chick.¹ It is apparent from such a review that investigators have differed widely as to the exact sources and the histogenesis of this ganglion. According to Hoffmann ('85), Ewart ('90) and Chiarugi ('94, '97), the ciliary ganglion arises from cells which migrate from the mesocephalic region of the semilunar ganglion into the oculomotor nerve either directly or by way of the ophthalmic division of the trigeminal nerve. According to Dohrn ('91), the ciliary ganglion arises in the selachians from cells which wander out from the mid-brain along the path of the oculomotor nerve. Béraneck ('84), Reuter ('97) and Rex ('00) also derive the ciliary ganglion from cells present in the oculomotor nerve but do not determine the exact sources of these cells.

According to Carpenter's observations, the ciliary ganglion in the chick arises primarily from cells which advance peripherally from the wall of the mid-brain along the oculomotor nerve; a few cells being added which advance peripherally from the semilunar ganglion by way of the ophthalmic nerve. These latter cells, according to Carpenter, are easily distinguished from the cells which enter the ciliary ganglion by way of the oculomotor nerve by reason of the larger size of their nuclei and the greater abundance of their cytoplasm. He, therefore, recognizes two distinct regions in the ciliary ganglion.

Regarding the sphenopalatine, the otic and the submaxillary ganglia, the evidence at hand seems to favor the theory that they are derived exclusively from the semilunar ganglion.

The present investigation was undertaken in order to extend the writer's earlier observations on the development of the sympathetic nervous system in the vertebrate series and to correlate the cranial sympathetic ganglia with the other parts of the sympathetic nervous system.

¹ Bulletin of the Museum of Comparative Zoology at Harvard College, vol. 48, pp. 141-229.

The following observations are based almost exclusively on embryos of the pig. As in my earlier investigations of the development of the sympathetic nervous system,² the most satisfactory preparations were obtained from embryos which were fixed in chrom-aceto-formaldehyde, cut to a thickness of 10 μ and stained by the iron-hematoxylin method. This method was employed almost exclusively in the present investigation. Sagittal, or parasagittal sections were found to be most serviceable for the study of the cranial sympathetic ganglia and were used almost exclusively.

I take pleasure in expressing my sense of obligation to Prof. G. L. Houser for valuable suggestions during the progress of this investigation. I desire also to express my indebtedness to Prof. F. A. Stromsten for material assistance in technique.

OBSERVATIONS

Introductory

The cranial sympathetic ganglia are genetically related to the oculomotor and the trigeminal nerves. A study of the development of the former, therefore, involves a study of the early development of the latter. The relation of the cranial sympathetic ganglia to the oculomotor and the several divisions of the trigeminal nerves is illustrated in the accompanying figure (fig. 1) which is drawn semidiagrammatically from a wax reconstruction of the third and the fifth cranial nerves in an embryo of the pig 27 mm. in length.

Trigeminal nerve

The trigeminal nerve arises from the wall of the anterior region of the rhombencephalon by a relatively small motor root and a larger sensory root upon which is located the semilunar ganglion. The ophthalmic and the maxillary divisions of the trigeminal nerve arise as fibrous outgrowths from the semilunar ganglion. The motor root of the trigeminus takes part only in the formation of the mandibular division. In embryos of the pig 5 to

² See bibliography.

6 mm. in length, the neural crest has already become differentiated into ganglionic masses. At this stage the anlage of the semilunar ganglion appears as a somewhat irregular mass of cells lying in close proximity with the lateral surface of the anterior region of the rhombencephalon and almost or quite in contact with the ectoderm. As development advances the position of this ganglionic mass is shifted ventrad until the entire anlage lies ventro-lateral to the rhombencephalon in the region of the pons.

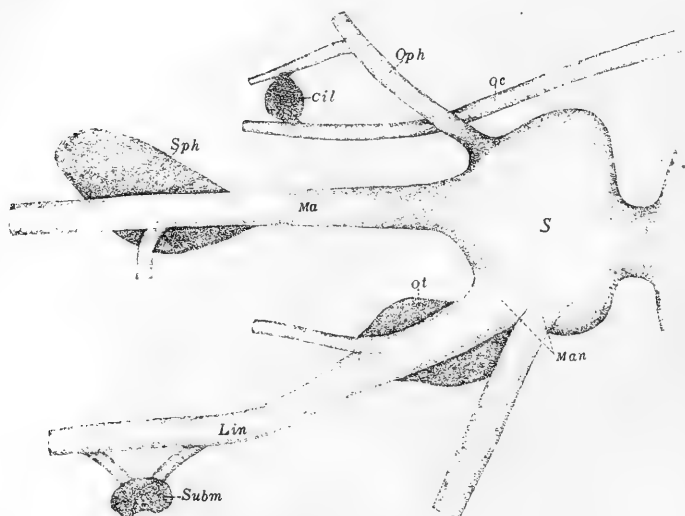


Fig. 1 Drawing made from a wax reconstruction of the third and the fifth cranial nerves in an embryo of the pig 27 mm. in length. *Cil*, ciliary ganglion; *Lin*, lingual nerve; *Ma*, maxillary nerve; *Man*, mandibular nerve; *Oc*, oculo-motor nerve; *Oph*, ophthalmic nerve; *Ot*, otic ganglion; *S*, semilunar ganglion; *Sph*, sphenopalatine ganglion; *Subm*, submaxillary ganglion.

In embryos of the pig 8 mm. in length, the semilunar ganglion is quite definitely outlined. It still lies in close proximity with the wall of the rhombencephalon. Its proximal surface is slightly concave, while its peripheral surface is irregularly convex. The entire mass, therefore, is already roughly crescentic in outline. The fibers of the sensory root of the trigeminal nerve have already penetrated the wall of the rhombencephalon and the motor root

is well differentiated. The nuclei of the cells composing the ganglionic mass are identical in appearance with the nuclei of the majority of the cells in the mantle layer in the neural tube. In general the nuclei in the ganglion are oriented so that their long axes are directed peripherally. This orientation, however, is by no means constant.

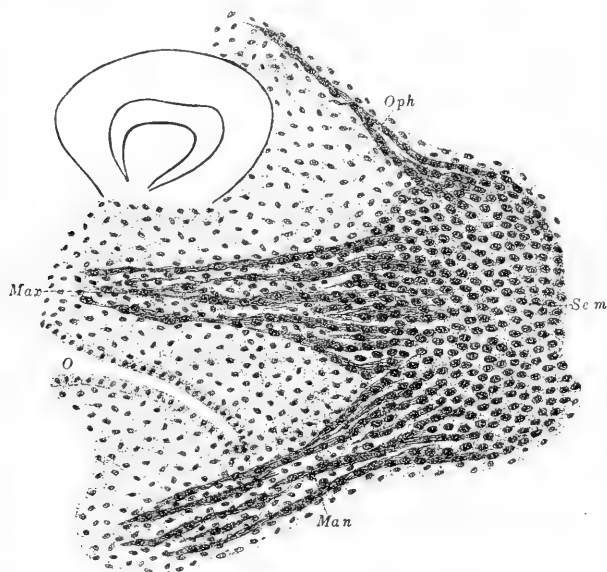


Fig. 2 Parasagittal section showing semilunar ganglion and the main divisions of the trigeminal nerve in an embryo of the pig 8 mm. in length. *Max*, maxillary nerve; *Man*, mandibular nerve; *O*, oral cavity; *Oph*, ophthalmic nerve; *Sem*, Semilunar ganglion.

In parasagittal sections of embryos of the pig 8 mm. in length, the three main divisions of the trigeminal nerve may be traced peripherally for a considerable distance (fig. 2). The ophthalmic division is composed of a slender bundle of fibers which may be traced anteriorly above the upper margin of the optic vesicle into the region of the orbit (fig. 2, *Oph*). The maxillary division is composed of loosely aggregated bundles of fibers which may be traced anteriorly above the oral cavity as far as the anterior margin of the optic cup (fig. 2, *Max*). This division emerges

from the middle part of the peripheral surface of the semilunar ganglion by a broad base and converges gradually toward its peripheral extremity. The mandibular, like the maxillary division, is composed of loosely aggregated fiber-bundles. This division may be traced from the ventral angle of the semilunar ganglion antero-ventrally into the mandibular region (fig. 2, *Man*).

The semilunar ganglion, during the early stages of development, is not sharply limited peripherally. Cells push out into the proximal parts of the nerves arising from its periphery so that in these regions it is quite impossible to determine the exact limits of the ganglionic mass. Similar cells may be observed associated with the fiber-bundles throughout the entire length of the nerve-trunks. It is obvious, therefore, that cells of nervous origin advance peripherally from the semilunar ganglion along the fibers of all three divisions of the trigeminal nerve.

The motor root of the trigeminal nerve does not penetrate the semilunar ganglion but advances diagonally ventrad and unites with the sensory root of the mandibular division of the trigeminal nerve. In embryos of the pig 10 to 11 mm. in length, the motor root of the trigeminal nerve may be traced from the wall of the rhombencephalon as a bundle of closely aggregated fibers (fig. 3, *MR*). The cells of the mantle layer at this point push out across the marginal veil into the proximal part of the nerve-root and many of them obviously migrate peripherally along its growing fibers. In many sections through this region continuous rows of medullary cells may be traced from the mantle layer in the rhombencephalon into the proximal part of this motor nerve-root (fig. 3, *MC*). Similar cells may be observed all along the fibers of this motor root as well as along the mandibular nerve peripheral to the point of union of the sensory and the motor roots. Beyond this point the cells of medullary and of ganglionic origin can not be distinguished from each other. It is probable, however, that cells from both these sources advance peripherally along the fibers of the mandibular nerve.

Oculomotor nerve

The oculomotor nerve emerges from the wall of the mesencephalon by several slender rootlets arranged in a longitudinal series (fig. 4, *Oc*). The proximal part of this nerve, in sagittal sections, therefore, appears distinctly fan-shaped. Throughout

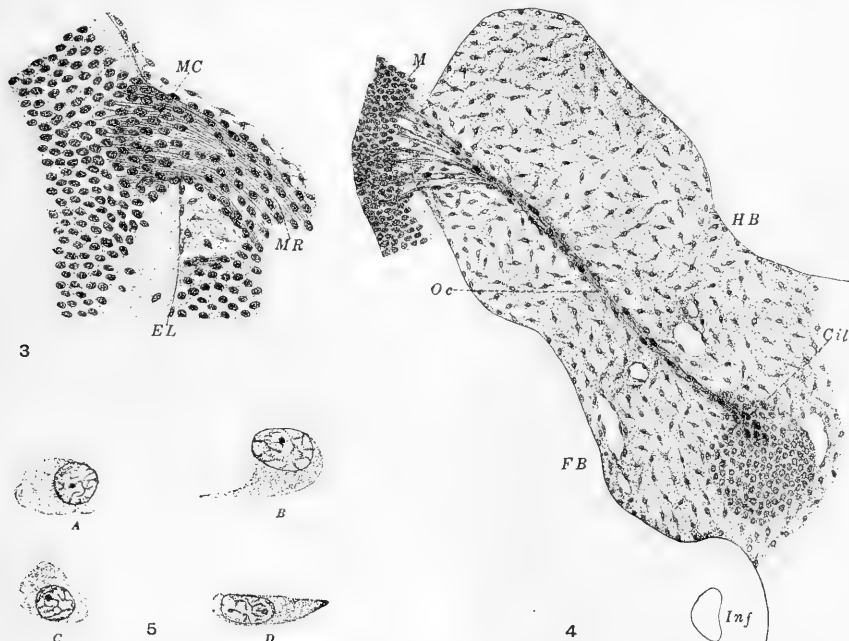


Fig. 3 Section showing the motor root of the trigeminal nerve in an embryo of the pig 11 mm. in length. *EL*, external limiting membrane; *MC*, migrant medullary cells; *MR*, motor root.

Fig. 4 Section showing the oculomotor nerve in an embryo of the pig 8 mm. in length. *Cil*, ciliary ganglion; *FB*, fore-brain; *Inf*, infundibulum; *HB*, hind-brain; *M*, mid-brain; *Oc*, oculomotor nerve.

Fig. 5 Neuroblasts (drawn under oil immersion lens with aid of camera lucida). *A*, observed in mesenchyme between ophthalmic nerve and ciliary ganglion, embryo 14 mm. in length, *B*, *C*, *D*, observed in ophthalmic nerve, embryo 14 mm. in length.

the entire length of the growing nerve, its fibers are accompanied by cells obviously of medullary origin. These cells are identical in appearance with the cells which, as indicated above, advance peripherally from the semilunar ganglion and from the wall of

the rhombencephalon along the several divisions of the trigeminal nerve. They are obviously cells which wander out from the mantle layer in the wall of the mesencephalon along the rootlets of the oculomotor nerve. In the early stages of development, as may be observed in parasagittal sections, medullary cells push out from the mantle layer in cone-shaped masses into the rootlets of the oculomotor nerve until they come into close proximity with the external limiting membrane. Continuous lines of medullary cells extending from the mantle layer into the proximal parts of the oculomotor nerve could never be observed. However, cells identical in appearance with the medullary cells are always present in the nerve-roots outside the external limiting membrane as well as throughout the entire length of the growing nerve. That these elements are cells of medullary origin which have wandered out from the mantle layer in the mesencephalon can not be doubted. They can not be traced from any other source. My observations on this point substantially confirm the observations of Carpenter ('06) on embryos of the chick and of other investigators on embryos of various types of vertebrates.

Migrant cells

As indicated above, the cells which advance peripherally from the semilunar ganglion and from the walls of the mesencephalon and rhombencephalon along the oculomotor nerve and the several divisions of the trigeminal nerve are identical, in the early stages of development, with the majority of the cells in the cerebrospinal ganglia and the mantle layer in the neural tube. In well stained preparations, these cells may be distinguished from the cells of the surrounding mesenchyme by the somewhat larger size, the more intense staining reactions and the characteristic chromatin structure of their nuclei. The great majority of them are characterized by very little cytoplasm and by a large rounded or elongated nucleus showing a very delicate chromatin structure. These cells are identical with the great majority of the cells which, as was previously pointed out by Carpenter and Main ('07) and as was shown by the writer in an earlier paper,³ advance

³ Anatomical Record, vol. 3, pp. 158-165.

peripherally from the neural tube and the spinal ganglia, in early embryos of the pig, along the spinal nerves. As the writer has shown in a series of earlier papers,⁴ the cells which thus advance peripherally from the cerebro-spinal nervous system in vertebrate embryos are the descendants of the 'germinal' cells (Keimzellen) of His, viz., the 'indifferent' cells and the 'neuroblasts' of Schaper. Nearly all of the cells advancing peripherally from the cerebro-spinal nervous system along the fibers of the growing nerves, in the early stages of development, are cells of the 'indifferent' type. These cells, as Schaper has pointed out, may give rise to neuroblasts or to embryonic supporting cells, or they may retain the capacity for further propagation by division and give rise to new generations of 'indifferent' cells. Occasional mitotic figures along the paths of the growing nerves indicate that some of these cells have retained the capacity for further propagation by division after they have become separated from the cerebro-spinal nervous system. Among the cells of the 'indifferent' type cells may occasionally be observed in the paths of the growing nerves which are obviously neuroblasts. These cells are characterized by a large cytoplasmic body which may or may not be drawn out to a point at one side and a large rounded or elongated nucleus showing little structure in the interior except a well defined nucleolus (fig. 5).

As the cells of the 'indifferent' type advance peripherally along the growing nerves the nuclei of many of them become distinctly elongated or more or less irregular in outline. Nuclei may be observed occasionally which are distinctly pyriform with the broader end directed peripherally. Such modifications in the form of the nuclei of the cells present in the growing nerves during the early stages of development are, doubtless, correlated with the processes involved in their peripheral displacement. The nuclei which remain associated with the nerve-fibers, during the later stages of development, become extremely elongated. These, however, are the nuclei of the cells which are obviously becoming differentiated to form the neurilemma.

⁴ See bibliography.

Ciliary ganglion

In embryos of the pig 8 mm. in length, as shown in parasagittal section in figure 4, the oculomotor nerve terminates among the closely aggregated mesenchyme cells which constitute the anlage of the posterior rectus muscle. A few cells of nervous origin which have advanced peripherally along the path of this nerve may be observed aggregated at its growing tip (fig. 4, *Cil*). These cells, doubtless, constitute the anlage of the ciliary ganglion.

In embryos 12 to 14 mm. in length, the oculomotor nerve has advanced farther peripherally into the region of the future orbit. The anlage of the ciliary ganglion now appears as a small aggregate of cells lying in more or less intimate contact with the oculomotor nerve a short distance posterior and ventro-mesial to the optic cup (fig. 6, *Cil*). In parasagittal sections of an embryo 14 mm. in length which cut the ophthalmic nerve longitudinally, oblique sections of the oculomotor nerve may be observed distal to the periphery of the semilunar ganglion and between the ophthalmic and the maxillary divisions of the trigeminal nerve (fig. 6, *Oc*). In such sections, as shown in figure 6, the anlage of the ciliary ganglion lies in close proximity with the path of the oculomotor nerve. Between this anlage and the ophthalmic nerve, a few small groups of cells obviously of nervous origin may be observed lying in the mesenchyme (fig. 6, *MC*). These cells, doubtless, represent nervous elements advancing from the ophthalmic nerve toward the anlage of the ciliary ganglion. In one instance two of the cells contained in one of these groups were obviously neuroblasts. One of these cells is illustrated in figure 5, *A*. A few neuroblasts were observed also in the ophthalmic nerve. The presence of a slight accumulation of nervous element at the ventral side of the ophthalmic nerve and of groups of similar cells lying in the mesenchyme approximately in a direct line from this point toward the anlage of the ciliary ganglion warrants the conclusion that cells which advance peripherally from the semilunar ganglion along the fibers of the ophthalmic nerve deviate from the course of this nerve and, advancing through the mesenchyme, enter the anlage of the

ciliary ganglion. At a later stage, as will be shown presently, the ciliary ganglion becomes connected with the ophthalmic nerve by a fibrous ramus.

In embryos of the pig 20 to 21 mm. in length in which the eye-muscles are already well differentiated, the ciliary ganglion lies in close proximity with the optic stalk, but still remains intimately associated with the oculomotor nerve (fig. 7, *Cil*). Fibers from this nerve now penetrate the ganglionic mass. The ciliary ganglion, up to this stage of development, is much more intimately associated with the oculomotor than with the oph-

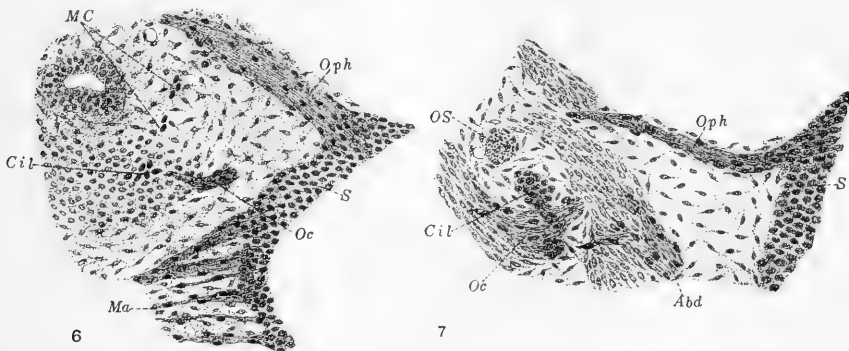


Fig. 6 Parasagittal section cutting ophthalmic nerve longitudinally, embryo of pig 14 mm. in length. *Cil*, ciliary ganglion; *Ma*, maxillary nerve; *MC*, migrant cells; *Oc*, oculomotor nerve; *Oph*, ophthalmic nerve; *S*, semilunar ganglion.

Fig. 7 Parasagittal section cutting ophthalmic nerve longitudinally, embryo of pig 21 mm. in length. *Abd*, abducent nerve; *Cil*, ciliary ganglion; *Oc*, oculomotor nerve; *Oph*, ophthalmic nerve; *OS*, optic stalk; *S*, semilunar ganglion.

thalmic nerve. While there can be no doubt that some cells which advance peripherally from the semilunar ganglion along the ophthalmic nerve enter the ciliary ganglion, it is probable that the great majority of the cells which become incorporated in this ganglion are cells which advance peripherally along the oculomotor nerve. At a later stage, as illustrated in figure 8, which is taken from a parasagittal section of an embryo of the cat 22 mm. in length, the ciliary ganglion is connected with the ophthalmic nerve by a fibrous ramus. It is improbable, however, that many nervous elements advance peripherally after this connection is established.

As development advances, the cells which have advanced peripherally and become incorporated in the ciliary ganglion increase in size less rapidly than the cells which remain in the cerebro-spinal nervous system. In embryos 15 to 20 mm. in length in which the ciliary ganglion is already well established and many of its constituent cells have become differentiated into neuroblasts, the cells composing this ganglion are materially smaller than the cells in the semilunar ganglion.

Carpenter ('06), in his study of the ciliary ganglion in the chick, observed a constant difference in size between the migrant nervous elements in the ophthalmic nerve and the cells in the anlage of the ciliary ganglion; the former being materially larger than the latter. He also observed a few cells of the larger variety in the ciliary ganglion. These observations suggested to this author an intrinsic difference between the cells which enter the anlage of the ciliary ganglion by way of the oculomotor and the ophthalmic nerves respectively. As indicated above, the cells in the ciliary ganglion, in advanced embryos of the pig, are materially smaller than the cells in the semilunar ganglion. However, I have never observed any difference in the size or appearance of the nervous elements which have become incorporated in the ciliary ganglion, in embryos of the pig, which would suggest an intrinsic difference in these elements.

Sphenopalatine ganglion

The sphenopalatine ganglion arises as an irregular mass of loosely aggregated cells lying along the median surface of the maxillary nerve. In the early stages of development, as illustrated in figure 2, the maxillary nerve is composed of many small loosely aggregated bundles of fibers accompanied by numerous cells of ganglionic origin. These cells push out from the periphery of the semilunar ganglion in cone-shaped aggregates into the proximal part of this nerve and, becoming completely separated from the ganglionic mass, many of them advance peripherally along the growing nerve.

The fiber-bundles composing the maxillary nerve remain loosely aggregated until comparatively late in the course of develop-

ment. In parasagittal sections of embryos 12 to 15 mm. in length, small groups of cells of ganglionic origin may be observed along the median surface of the maxillary nerve. Some of these cell-groups lie distinctly within the path of the nerve, while others lie in close proximity with its surface. These loosely aggregated cell-groups represent the anlage of the sphenopalatine

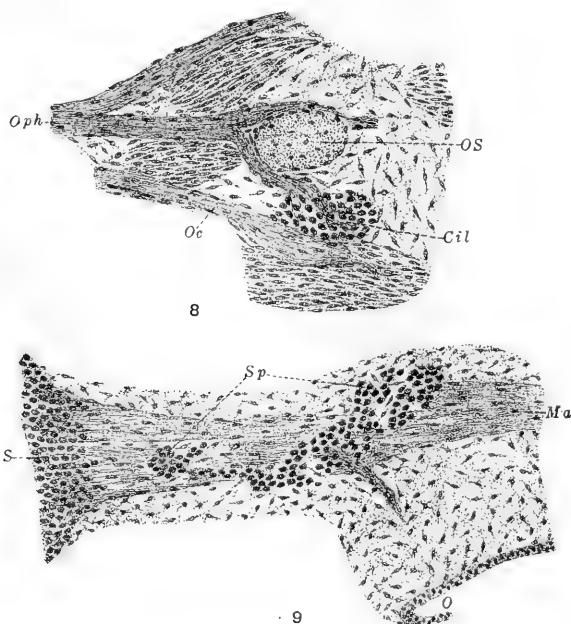


Fig. 8 Parasagittal section through orbit, embryo of cat 22 mm. in length. *Cil*, ciliary ganglion; *Oc*, oculomotor nerve; *Oph*, ophthalmic nerve; *OS*, optic stalk.

Fig. 9 Parasagittal section near median surface of maxillary nerve, embryo of pig 19 mm. in length. *Ma*, maxillary nerve; *O*, oral cavity; *S*, semilunar ganglion; *Sp*, sphenopalatine ganglion.

ganglion. As development advances, these cell-groups become larger and push out farther peripherally along the path of the growing nerve. In embryos of the pig 19 mm. in length, as illustrated in figure 9, *Sp*, an irregular mass of loosely aggregated cells is observed beginning but a short distance from the periphery of the semilunar ganglion and stretching along the median

surface of the maxillary nerve to a point peripheral to the anterior border of the orbit. A considerable portion of the ganglionic mass, as shown in figure 9, lies above the dorsal level of the nerve-trunk.

The cells which accompany the fibers of the maxillary nerve during the early stages of development, as well as the first cells which become aggregated to form the anlage of the sphenopalatine ganglion, are identical in appearance with the cells which remain in the semilunar ganglion. As development advances, however, the cells which remain in the semilunar ganglion increase in size more rapidly than the cells which have advanced peripherally. The cells composing the anlage of the sphenopalatine ganglion are, therefore, materially smaller than the cells in the semilunar ganglion. If the former were compared with the latter at this time their nervous character might be doubted. The cells in the sphenopalatine ganglion soon begin to increase in size more rapidly, however, and many of them rapidly become differentiated into neuroblasts.

In embryos 25 to 27 mm. in length, the proximal part of the ganglionic mass described in the preceding stage as stretching along the median surface of the maxillary nerve has advanced farther peripherally. The entire ganglionic mass is now more closely aggregated and a large portion of it still lies above the dorsal level of the nerve-trunk. This condition is illustrated in figure 10 which is taken from a parasagittal section of an embryo of the pig 27 mm. in length. After this stage, the entire ganglionic mass becomes more compactly aggregated and its position is shifted ventrally until a portion of the ganglion lies below the ventral level of the nerve-trunk and partly or completely surrounds the proximal part of a descending branch which arises at this point.

According to the above observations, the sphenopalatine ganglion is derived more or less directly from the semilunar ganglion. It becomes connected, in the course of development, with the geniculate ganglion of the facial nerve by the large superficial petrosal nerve. This connection, however, is not made until the anlage of the sphenopalatine ganglion is well established. The



Fig. 10 Photomicrograph of parasagittal section near median surface of maxillary nerve, embryo of pig 27 mm. in length. *Ma*, maxillary nerve; *S*, semilunar ganglion; *Sp*, sphenopalatine ganglion.

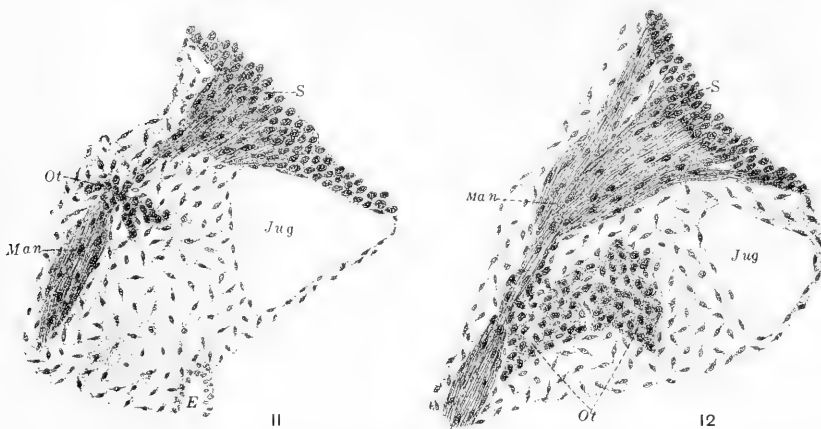


Fig. 11 Parasagittal section near median surface of mandibular nerve, embryo of pig 17 mm. in length. *E*, eustachian tube; *Jug*, jugular vein; *Man*, mandibular nerve; *Ot*, otic ganglion; *S*, semilunar ganglion.

Fig. 12 Parasagittal section near median surface of mandibular nerve, embryo of pig 21 mm. in length. *Jug*, jugular vein; *Man*, mandibular nerve; *Ot*, otic ganglion; *S*, semilunar ganglion.

possibility is not precluded that a few cells which wander out from the geniculate ganglion along the path of the large superficial petrosal nerve may become incorporated in the sphenopalatine ganglion. It is improbable, however, that any considerable number of cells is contributed to the sphenopalatine ganglion from this source.



Fig. 13 Photomicrograph of parasagittal section near median surface of mandibular nerve, embryo of pig 27 mm. in length. *Man*, mandibular nerve; *Ot*, otic ganglion; *S*, semilunar ganglion.

Otic ganglion

The otic ganglion arises in embryos of the pig as an irregular mass of cells of ganglionic and of medullary origin at the median surface of the proximal part of the mandibular division of the trigeminal nerve. Like the maxillary division of the trigeminal nerve, the mandibular division, during the early stages of development, is composed of a large number of loosely aggregated bundles of fibers which are accompanied by numerous cells of nervous origin. The mandibular nerve, unlike the maxillary, arises by two roots, viz., a sensory root which emerges from the ventro-lateral aspect of the semilunar ganglion and a motor root which grows out directly from the wall of the rhombencephalon. These two roots unite immediately peripheral to the semilunar

ganglion. As indicated above and illustrated in figure 3, cells of medullary origin wander out from the mantle layer in the wall of the rhombencephalon into the motor root of the trigeminal nerve and advance peripherally along its fibers. The cells of nervous origin accompanying the fibers of the mandibular nerve are, therefore, derived from two sources, viz., the rhombencephalon and the semilunar ganglion.

In parasagittal sections of embryos of the pig 12 to 15 mm. in length which pass close to the median surface of the proximal part of the mandibular nerve, small groups of cells may be observed which are removed by only a short interval from the periphery of the semilunar ganglion and are closely associated with the growing fibers of the mandibular nerve. These cell-groups constitute the anlage of the otic ganglion. In similar sections of embryos 17 mm. in length, as illustrated in figure 11, *Ot*, these aggregates of cells have become larger and are somewhat farther removed from the periphery of the semilunar ganglion. The ganglionic anlage now appears as an irregular mass of cells which is closely associated with the median surface of the mandibular nerve. In embryos 21 mm. in length, the anlage of the otic ganglion has increased materially in size and the major portion of it lies distinctly below the path of the nerve and is removed by only a short interval from the jugular vein (fig. 12, *Ot*).

In embryos of the pig 25 to 27 mm. in length, the cells composing the anlage of the otic ganglion have become more compactly aggregated and the entire ganglionic mass appears correspondingly smaller and more closely associated with the nerve-trunk (fig. 13, *Ot*). Many of the cells in the ganglion may now be recognized as neuroblasts. The ganglion remains more or less irregular in outline and from its postero-ventral aspect a cord of cells associated with a few fibers may be traced toward the parotid gland. This condition was observed also in an embryo of the cat 22 mm. in length.

As in the case of the sphenopalatine ganglion, the cells which constitute the earliest anlage of the otic ganglion are identical in appearance with the cells in the semilunar ganglion. As devel-

opment advances, however, the cells in the semilunar ganglion increase in size more rapidly than those which have advanced farther peripherally. Consequently, during the later stages of development, the cells in the otic ganglion appear materially smaller than those in the semilunar ganglion. This disparity in the size of the cells in the otic ganglion, as in the case of the sphenopalatine ganglion, becomes less marked after they have become differentiated into neuroblasts.

During the later stages of development, fibers may be traced from the mandibular nerve into the otic ganglion. These fibers, doubtless, give rise to both the secretory and the sensory short roots of the adult ganglion. In the course of development, the otic ganglion becomes connected also with the facial and the glossopharyngeal nerves by the small superficial petrosal nerve. The delicate sympathetic root which in the adult condition connects the otic ganglion with the sympathetic plexus on the middle meningeal artery could not be observed in my preparations.

As already indicated, cells advance peripherally both from the semilunar ganglion and from the wall of the rhombencephalon along the path of the mandibular nerve. That cells which advance peripherally from the semilunar ganglion enter the anlage of the otic ganglion can not be doubted. Inasmuch as the motor root of the trigeminal nerve unites with the mandibular division just proximal to the otic ganglion, it is highly probable that many cells which wander out from the wall of the rhombencephalon along the fibers of this motor root also become incorporated in this ganglion. It is probable, therefore, that the otic ganglion receives cells from both the semilunar ganglion and the nidulus of the motor root of the trigeminal nerve. Perhaps all the nervous elements which take part in the development of the otic ganglion are derived from these two sources. The relationships of the small superficial petrosal nerve to the facial and the glossopharyngeal nerves render it highly improbable that any cells which wander out along the path of the former are contributed to the otic ganglion. Furthermore, the anlage of the otic ganglion is well established before any trace of the small

superficial petrosal nerve can be found. Likewise, it is highly improbable that any cells are contributed to the otic ganglion by way of its sympathetic root.

Submaxillary ganglion

The submaxillary ganglion arises as an accumulation of cells of nervous origin associated with the lingual division of the mandibular nerve. In embryos of the pig 13 to 16 mm. in length, the mandibular division of the trigeminal nerve is already differentiated into its component parts. In parasagittal sections of embryos at this stage, the lingual and the inferior dental divisions of the mandibular nerve may be traced into the mandibular region. The lingual nerve passes lateral to the pharynx and beneath the root of the tongue where it may be traced anteriorly in the floor of the oral cavity well toward the tip of the mandible. Just beneath the root of the tongue the lingual nerve gives rise to several slender branches which grow ventro-mesially. Cells which advance peripherally along the lingual nerve wander out along one or more of these slender branches and become aggregated to give rise to the anlage of the submaxillary ganglion. The anlage of the submaxillary ganglion, therefore, arises in contact with one or more of these slender branches and is removed by only a short interval from the path of the lingual nerve.

The relationships of the submaxillary ganglion to the lingual nerve, in the early stages of development, can not be well illustrated in drawings made from sections because of the somewhat irregular course of the lingual nerve and its branches. Figure 14 represents a parasagittal section passing through the anlage of the submaxillary ganglion in an embryo of the pig 16 mm. in length. The ganglionic anlage in this instance is removed from the lingual nerve (not shown in the section) by a short interval and is located at the extremity of a slender branch along which its component cells have obviously wandered out from the nerve-trunk.

In embryos 20 to 21 mm. in length, the ganglionic mass has increased materially in size and lies in contact distally with a

mass of closely aggregated mesenchyme cells which constitute the anlage of the submaxillary gland. Figure 15 represents a parasagittal section through the anlage of the submaxillary ganglion in an embryo 21 mm. in length. In this instance the ganglionic

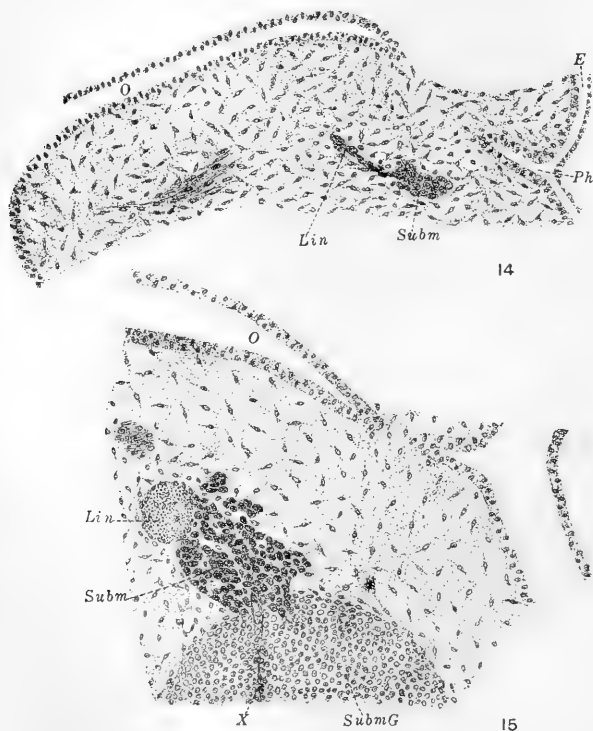


Fig. 14 Parasagittal section through anlage of submaxillary ganglion, embryo of pig 16 mm. in length. *E*, eustachian tube; *Lin*, lingual nerve; *O*, oral cavity; *Ph*, pharynx; *Subm*, submaxillary ganglion.

Fig. 15 Parasagittal section through anlage of submaxillary ganglion, embryo of pig 21 mm. in length. *Lin*, lingual nerve; *O*, oral cavity; *Subm*, submaxillary ganglion; *SubmG*, submaxillary gland; *X*, fibers accompanied by ganglionic cells.

anlage lies in contact proximally with the lingual nerve which is shown in oblique section in the figure and distally with the anlage of the submaxillary gland. A slender fiber-bundle accompanied by a few cells which have obviously wandered down from the ganglionic mass may be traced into the anlage of the submaxillary

gland (fig. 15, X). These cells may have been carried into the anlage of the gland more or less accidentally or they may be destined to take part in the development of the minute multiple ganglia which are known to exist in the substance of the submaxillary gland along the courses of its ducts.

As in the case of the other cranial sympathetic ganglia, the cells composing the anlage of the submaxillary ganglion, in the earliest stages of development, show the same general characters as the cells in the cerebrospinal ganglia. During the succeeding stages of development, however, the cells in the submaxillary ganglion, like those in the other cranial sympathetic ganglia, remain somewhat smaller than the nervous elements in the cerebrospinal nervous system and do not again betray their obvious relationships with the latter until they have become differentiated into neuroblasts.

In not a few instances isolated groups of cells of nervous origin were observed closely associated with the branches of the lingual nerve which supply the tongue, as well as in the path of the lingual nerve peripheral to the submaxillary ganglion. I was not able to determine the fate of these minute isolated ganglionic cell-groups. It seems probable that some of them may become aggregated to form a sublingual ganglion. In embryos of the pig 25 to 30 mm. in length, many of these cell-groups still remain isolated and some of the cells have become differentiated into neuroblasts.

During the earliest stages of its development, the submaxillary ganglion has fibrous connections only with the lingual nerve. This nerve, which is one of the main divisions of the mandibular nerve, doubtless, contains fibers from both the sensory and the motor root of the latter. As already indicated, cells advance peripherally from the semilunar ganglion and from the rhombencephalon along the sensory and the motor roots respectively of the mandibular nerve. It is probable, therefore, that cells from both these sources advance peripherally along the lingual nerve and become incorporated in the submaxillary ganglion. It is also probable that these are the sources of all the cells which enter the anlage of the submaxillary ganglion. The lingual nerve

becomes connected, in the course of development, with the facial nerve by means of the chorda tympani. The possibility is not precluded, therefore, that cells which wander out from the geniculate ganglion and advance peripherally along this communicating branch might be carried into the submaxillary ganglion. In view of the fact, however, that this connection is established comparatively late in the course of development it is quite improbable that cells are contributed to the submaxillary ganglion from this source. Likewise, it is improbable that any cells are contributed to the submaxillary ganglion by way of its sympathetic root which connects it with the sympathetic plexus on the facial artery.

CONCLUSIONS

In a series of earlier papers,⁵ the writer has shown that the ganglia of the sympathetic trunks and the prevertebral sympathetic plexuses arise from cells which have their origin in the spinal ganglia and the neural tube and advance peripherally along the sensory and the motor roots respectively of the spinal nerves. Likewise, the vagal sympathetic plexuses, including primarily the myenteric and the submucous plexuses, the pulmonary plexuses and the cardiac plexus, arise from cells which have their origin in the vagus ganglia and the walls of the hind-brain and advance peripherally along the paths of the vagi. The cells which advance peripherally from the cerebro-spinal nervous system along the spinal and the vagus nerves and give rise to the sympathetic nervous system are the descendants of the 'germinal' cells of His, viz., the 'indifferent' cells and the 'neuroblasts' of Schaper. The sympathetic nervous system, therefore, not only bears a genetic relationship to the cerebro-spinal nervous system, but is homologous with the other functional divisions of the nervous system.

The observations set forth in the preceding pages show clearly that the ciliary, the sphenopalatine, the otic and the submaxillary

⁵ See bibliography.

ganglia arise, in embryos of the pig, from cells which have their origin in the semilunar ganglion and the walls of the mesencephalon and rhombencephalon and advance peripherally along the oculomotor and the several divisions of the trigeminal nerves. These cells, like the cells which give rise to the other parts of the sympathetic nervous system, have their origin in a cerebro-spinal ganglion, i.e., a ganglion which is derived from the neural crest, and in motor noduli in the wall of the neural tube and advance peripherally along sensory and motor nerve-fibers respectively. The oculomotor and the trigeminal nerves, therefore, sustain the same genetic relationship to the cranial sympathetic ganglia as do the spinal nerves to the ganglia of the sympathetic trunks and the prevertebral sympathetic plexuses and the vagi to the vagal sympathetic plexuses. Furthermore, the cells which give rise to the cranial sympathetic ganglia, like the cells which give rise to the other parts of the sympathetic nervous system, are the descendants of the 'germinal' cells of His, viz., the 'indifferent' cells and the 'neuroblasts' of Schaper. These ganglia, therefore, arise in an analogous manner and bear the same genetic relationships to the cerebro-spinal nervous system as do the other parts of the sympathetic nervous system. Ontogenetic evidence, therefore, warrants the conclusion that these ganglia are sympathetic in character.

As is well known, the sympathetic character of the ciliary ganglion has been questioned by not a few investigators. It is of interest to note, therefore, that the ontogenetic evidence for the sympathetic character of the ciliary, the sphenopalatine, the otic and the submaxillary ganglia presented in this paper is in full accord with the recent histological observations of Müller and Dahl ('10) who find, in several mammalian types, that all of these ganglia are composed exclusively of multipolar neurones which do not differ materially from the sympathetic neurones in the other parts of the sympathetic nervous system.

SUMMARY

1. The ciliary ganglion arises from cells which advance peripherally from the mesencephalon and the semilunar ganglion respectively along the oculomotor and the ophthalmic nerves. The earliest anlage of the ciliary ganglion arises in contact with the oculomotor nerve. The majority of the cells which become incorporated in the ciliary ganglion, doubtless, advance peripherally along this nerve. These observations substantially confirm the observations of Carpenter ('06) on the development of the ciliary ganglion in the chick.

2. The sphenopalatine ganglion arises along the median surface of the maxillary nerve from cells which advance peripherally from the semilunar ganglion.

3. The otic ganglion arises at the median surface of the proximal part of the mandibular division of the trigeminal nerve from cells which advance peripherally from the semilunar ganglion and from the wall of the rhombencephalon respectively along the sensory and the motor roots of the mandibular nerve.

4. The submaxillary ganglion arises in the mandibular region in proximity with the lingual division of the mandibular nerve from cells which wander out from the semilunar ganglion and the wall of the rhombencephalon respectively along the sensory and motor roots of the mandibular nerve and advance peripherally along the lingual division.

5. The oculomotor and the trigeminal nerves sustain the same genetic relationship to the cranial sympathetic ganglia as do the spinal nerves to the ganglia of the sympathetic trunks and the prevertebral sympathetic plexuses and the vagi to the vagal sympathetic plexuses.

6. The ciliary, the sphenopalatine, the otic and the submaxillary ganglia arise in an analogous manner and bear the same genetic relationships to the cerebro-spinal nervous system as do the other parts of the sympathetic nervous system. Ontogenetic evidence, therefore, warrants the conclusion that these ganglia are sympathetic in character.

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NERVUS TERMINALIS IN REPTILES AND MAMMALS¹

J. B. JOHNSTON

From the Institute of Anatomy, University of Minnesota

TWELVE FIGURES

The table on the following pages will show in the briefest form the occurrence and relations of the nervus terminalis in various vertebrates.

In all the forms in which the nerve enters the olfactory bulb it has been shown that its fibers pierce the formatio olfactoria to pass on to their proper endings in some part of the forebrain. Where the nerve is described as entering the brain near the pre-optic recess it is quite probable that the point of entrance is near the neuroporic recess as in selachians. In both cases at any rate the root has its attachment to the brain beside the lamina terminalis. From these facts it appears that the nervus terminalis in most fishes and amphibians is a ganglionated nerve whose root enters the forebrain caudal to the olfactory bulb, usually near the site of the embryonic neuropore, and whose fibers are distributed to the wall of the nasal sac. The presence of bipolar ganglion cells in the course of the nerve shows that it is in part at least a receptive nerve. Whether there are also efferent fibers of the sympathetic type (vaso-motor) or other components in the nerve can not be determined at present.

The purpose of this paper is to give a general description of the nerve as it appears in certain reptilian and mammalian embryos. I wish to acknowledge my great obligation to Dr. G. Carl Huber who has kindly given me the free use of his excellent collection of human and other embryos. The embryos studied in his laboratory are indicated below.

¹Neurological Studies from the Institute of Anatomy, University of Minnesota, No. 17.

AUTHOR	FORMS STUDIED	POSITION OF ROOT	GANGLION	CENTRAL RELATIONS	DISTRIBUTION
Fritsch '78.....	Galeus canis	dorsal surface of telencephalon			
Pinkus '94, '95.....	Protopterus	rec. praeropticus			nasal sac near naris
Allis '97.....	Amia calva	bulbus olfactorius	diffuse		nasal epithelium far forward
Locy '99.....	Squalus embryos	dorsally near neuro-pore	near root	septum	lateral part of nasal sac
Sewertzoff '02.....	Ceratodus embryos	near rec. praeropt.	present		nasal sac, mucous membrane, not olfactory
Locy '03, '05.....	selachians		always present	septum	nasal sac
K. Fürbringer, '04..	adult Ceratodus	dorsal or ventral			
Bing and Burckhardt '04, '05.....	adult Ceratodus				
DeVries '05.....	human foetus 3 to 4 months	medial surface of lobus olfactorius	present		
Brookover '08, '10..	Amia calva	bulbus olfactorius	composed chiefly of sympath. cells		reticulum beneath olfactory epithelium; blood vessels
Sheldon '09.....	Cyprinus carpio	not seen	present	decussates in com. ant.	olfactory epithelium
Herrick '09.....	Rana pipiens	ventral, caudal to for. bulbaris	present	nuc. medianus	
Döllken '09.....	rabbit, mouse and human embryos	at conrescentia primitiva	present	septi	
Johnston '11.....	selachians	near rec. neurop. always		septum, gyrus fornicatus	vomero-nasal organ

AUTHOR	FORMS STUDIED	POSITION OF ROOT	GANGLION	CENTRAL RELATIONS	DISTRIBUTION
Brookover and Jackson '11..... McKibben '11.....	Anciurus embryos urodeles	bulbus olfactorius bulbus olfactorius	like that in <i>Amia</i> not seen	com. anterior nuc. praeopticus, hypothalamus, interpeduncu- lar region	
	turtle embryos	medial surface hem- isphere caudal to bulbus olfactorius	in several clumps on trunk and rami	toward com. an- terior	Rostral part of me- dial diverticulum of nasal sac
Present paper.....	pig and sheep en- bryos	at fissura prima cau- dal to bulbus olfac- torius	compact on root		vomero-nasal organ
	human embryos	at fissura prima cau- dal to bulbus ol- factorius	at first compact, later diffuse		with septal strands of olfactory nerve

PIG EMBRYOS

The ganglion of the nervus terminalis (ganglion terminale) is distinguishable in the 9 mm. pig and the nerve has been seen in all the specimens examined of various stages up to 53 mm. It is most conspicuous in embryos of 15 to 25 mm. after which it seems to grow smaller. It was not seen in the later stages examined, namely, of 74 mm. and 90 mm.

In the 22 mm. embryo (fig. 1) the root of the nerve enters the brain at the ventral (rostral) end of the fissura prima (His). The root fibers are traceable for some distance caudally, i.e., toward the anterior commissure. In figure 1 only the portion of the nerve which contains ganglion cells has been modelled. The elongated ganglion terminale extends rostrally along the medial side of the root of the olfactory nerve and continues for some distance along the four primary branches into which the nerve divides. From each of the free ends of the ganglion shown in the model, strands of nerve fibers extend peripherally. In this embryo the ganglion terminale is independent of the mass of cells lying among the root bundles of the olfactory nerve known to older authors as the 'olfactory ganglion.' In other embryos the two masses of cells are frequently more closely related (fig. 5). The ganglion terminale may easily have been seen by earlier workers and considered by them to be a part of the so-called olfactory ganglion. The cells of the latter have the appearance of neurilenoma cells, being slender or flattened cells taking a moderate stain in carmine. The cells of the ganglion terminale have larger, more globular nuclei, are more closely packed and take a deeper stain.

The peripheral branches of the nerve in this 22 mm. embryo pass down through the septum in several strands which end in the wall of the vomero-nasal organ and in a small area of the wall of the nasal sac immediately adjacent.

The peripheral course of the nerve is much more readily followed in sagittal sections. A model made from sagittal sections of a 15 mm. embryo is drawn in figure 3. Here the root of the nerve occupies the same position as in the previous embryo.

The ganglion is more compact and lies in the angle between the olfactory bulb and the forebrain proper. Peripherally the strands of the nerve pass through a depression in the medial wall of the olfactory sac, diverge a little from one another and converge again slightly to end in the wall of the vomero-nasal organ. One strand passes to the nasal sac rostral to the vomero-nasal organ.

In figure 2 are shown the ganglion and root as they appear in sagittal section of a 19 mm. embryo. The root fibers as they pass caudad in the brain wall are accompanied by slender elongated cells.

In the 53 mm. embryo the differentiation of gray masses in the brain has gone far enough to make it possible to determine the general relations of the root. In figure 6 A, which represents a transverse section of the rostral end of the forebrain, the medio-dorsal cortex is seen extending down in the medial wall of the hemisphere and ending abruptly. The lower portion of this medial cortex is the developing hippocampus. Below it is the precommissural or paraterminal body of Elliot Smith. The boundary line between the two corresponds to the zona limitans which separates the hippocampal primordium from the precommissural body in such primitive forms as selachians. In this section the root of the nervus terminalis is seen entering the brain a little below the border of the hippocampal cortex. The 16 mm. pig (fig. 5 A) shows the nerve just above the tuberculum olfactorium. As the writer has shown elsewhere ('11), the nervus terminalis in certain typical selachians follows the zona limitans and is lost in the brain substance near the neuroporic recess. It is therefore clear that the nervus terminalis enters the brain in the pig in the same relations as in selachians. Peripherally the nerve in the 53 mm. pig bears essentially the same relations as in the younger stage described. The ganglion is more slender and the nerve gives the appearance of being reduced in size actually as well as relatively.

I have not yet been fortunate in securing a Golgi impregnation of the root of this nerve in the pig. Peripherally it takes the silver impregnation fairly well and its distribution to the vomero-nasal organ has been verified by this method. A few bipolar

ganglion cells connected with its fibers have been stained also. Further, a single preparation shows fibers which leave the peripheral strands of this nerve just distal to its ganglion, to join the root of the olfactory nerve and enter the olfactory bulb. This shows that the peripheral strands which have been described as *nervus terminalis* contain also the fibers which persist in the adult as the vomero-nasal nerve.

THE SHEEP

In embryos of the sheep of about 15 and 20 mm. the *nervus terminalis* is clearly present. Its ganglion is somewhat less sharply defined but in general it presents the same relations as in pig embryos.

HUMAN EMBRYOS

In an embryo in the writer's collection (H. 16) of 15.3 mm. ganglion cells appear medial to the root of the olfactory nerve in a position corresponding to that of the *nervus terminalis* in the pig. There are groups of ganglion cells found also in the ventral and lateral parts of the olfactory nerve. They are distinguished from neurilemma cells by their larger size, globular nuclei and deeper stain. The bundles of the *nervus terminalis* can not be traced in this embryo but the position of these ganglion cells suggests that the *nervus terminalis* may be distributed in part to the lateral wall of the nasal sac.

In an embryo of 9.5 mm. the ganglion terminale can not be distinguished as it can be in pig embryos of the same length. This embryo was received very fresh and its fixation is excellent.

The embryo XLVII of the Huber collection, 31 mm. in length, is cut in faultless sagittal sections 15μ in thickness and stained in hematoxylin and congo red. A model has been made of a sufficient portion of the right half of the brain adjacent to the median plane to show the relations of the *nervus terminalis* (fig. 4). The olfactory bulb is well formed and the *fissura prima* is begun in the medial wall of the hemisphere. From the ventro-medial surface of the brain at the level of the *fissura prima*, and therefore behind the olfactory bulb, spring three small roots, two

of them more medially and in contact with one another, the third twelve sections farther laterally. The three roots unite upon the ventro-medial surface of the olfactory nerve and there enter a distinct small ovoid or pear-shaped ganglion terminale. From the distal border of this ganglion go off strands of nerve fibers which mingle with those strands of the olfactory nerve which run down in the nasal septum. The ganglion terminale is closely connected distally with these olfactory strands, which present the appearance of a network, and the nervus terminalis is lost at this point.

On the left side in this embryo only one root was seen, the ganglion was somewhat more diffuse and more intimately connected with the olfactory strands in the septum.

Two other embryos in the Huber collection, VI and XXXIII, each between 15 and 16 mm. in length, show the nervus terminalis, but less clearly than the 31 mm. embryo. A drawing from one of them is given in figure 7. The ganglion cells do not show as clearly in these sections as in the writer's embryo of the same stage (H. 16), but they supplement the latter by showing the root in the usual position.

A fourth embryo in the Huber collection, IXL, 47 mm., shows the relations of the nervus terminalis clearly. It arises from the hemisphere just caudal to the olfactory bulb (fig. 8), passes rostrad ventro-medial to the bulb, comes in contact with the rostromedial surface of the 'olfactory ganglion' on the distal end of the bulb, and then joins the strands of the olfactory nerve so that it could not be followed farther.

It should be emphasized that in both the 31 mm. and 47 mm. embryos it is perfectly clear that the nervus terminalis joins with numerous strands of the olfactory nerve to make up the network of nerve bundles in the septum nasale. The writer was unable to demonstrate that the nervus terminalis was restricted to the immediate vicinity of the vomero-nasal organ as is the case in the pig.

EMYS LUTARIA

Two embryos of this turtle were studied in Dr. Huber's laboratory. In embryo *F*, 10 mm. in length, the nervus terminalis arises from the rostral end of the medial wall of the hemisphere, caudal to the olfactory bulb, descends over the medial surface of the bulb and olfactory nerve and bears clumps of ganglion cells at several points of its course (fig. 9). It comes into close relation with the dorsal division of the olfactory nerve but is distinguishable from it.

The peripheral distribution of the nerve is clearer in the 20 mm. embryo (*K*), in which it becomes more closely related to the dorsal division of the olfactory nerve. As shown in figure 11, the formatio olfactoria in the bulb is divided into dorsal and ventral portions. The dorsal portion receives the dorsal division of the olfactory nerve and the appearances in the bulb alone strongly suggest comparison with the vomero-nasal nerve and the accessory bulb into which it enters in marsupials and mammals. When the dorsal division of the olfactory nerve is traced distally, however, it is found to take a peculiar course which is illustrated diagrammatically in figure 10. It remains distinct from the ventral division, passes downward in the septum over the wall of a medial diverticulum of the nasal sac, turns laterad beneath the sac and is distributed to the wall of the most lateral portion of the nasal sac, situated beneath the orbit. This course and distribution seems to preclude the possibility of comparing this dorsal division of the nerve with the vomero-nasal nerve of mammals. The medial diverticulum of the nasal sac in this embryo seems to be the beginning of the vomero-nasal organ, and this is innervated by the ventral division of the olfactory nerve and in part by the nervus terminalis (fig. 10).

The nervus terminalis appears in sagittal sections rather far dorsally in the medial wall of the hemisphere (fig. 12 A). It passes forward over the medial wall of the bulb, converging with its fellow until they touch where the tips of the olfactory bulbs approach closest to the median plane (fig. 12 C). The nerve then passes alongside the dorsal division of the olfactory nerve and becomes somewhat mingled with it. At intervals groups of

ganglion cells appear in the dorsal division, which the writer attributes to the nervus terminalis (fig. 12 D, E). As the olfactory nerve approaches the nasal sac, its ventral division spreads out to innervate the medial diverticulum as well as the dorso-lateral wall (fig. 10, 12 D). The dorsal division breaks up into several strands which pass downward over the medial surface of this diverticulum to run beneath the nasal sac and reach its extreme lateral portion as above described. Meantime several small strands leave the dorsal division and bend rostrad over a partly separate rostral portion of the medial diverticulum, to which they are distributed. These small strands bear clumps of ganglion cells (fig. 12 E) which mark them as strands of the nervus terminalis.

Taking both the 10 and 20 mm. embryos into account, it may be said that the ganglion terminale in *Emys* is scattered in clumps of variable size along the nerve from its root to the terminal branches near the nasal sac.

The foregoing description shows that in the embryos of certain reptiles and mammals the nervus terminalis enters the brain at a point somewhat removed from the median plane but otherwise holding the same relation to the primordium hippocampi, pre-commissural body and neuroporic recess which the root holds in selachians. Its fibers arise from bipolar ganglion cells which are collected into a compact ganglion terminale (pig) or are gathered into several clumps in the course of the nerve and its branches (turtle). In the pig the nervus terminalis is clearly distributed to the vomero-nasal organ and in the turtle to a medial diverticulum of the nasal sac which presumably corresponds to the vomero-nasal organ or a part of it. In man the fibers mingle with the olfactory strands of the nasal septum.

Two authors have dealt with the nervus terminalis in mammals, de Vries ('05) and Döllken ('09). De Vries' paper is not accessible to me, but from the references to it by other authors it appears that he recognized a vomero-nasal nerve and vomero-nasal ganglion whose root entered the medial surface of the rhinencephalon caudal to the bulbus olfactorius. He saw also ganglion cells scattered in the course of the nerve. He inter-

preted the entire structure as the equivalent of the nervus terminalis of fishes.

Döllken's paper has come into my hands since the present manuscript was finished and the figures drawn. Döllken's description of the nervus terminalis and ganglion terminale in mouse, rabbit, and human embryos is in essential agreement with the above account for the pig, sheep and man. The central connections of the root may be passed over briefly. Only Döllken's root *c* ending in the septum corresponds to the root seen by me. The continuity of nervus terminalis roots with fibers leading to the gyrus fornicatus and hippocampus seems to the writer to require confirmation. It is not clearly demonstrated in Döllken's figures.

Döllken apparently regards the nervus terminalis as the special nerve of the vomero-nasal organ. He describes a part of its fibers at least as arising from cells in the vomero-nasal epithelium. He uses the name ganglion terminale as synonymous with 'ganglion vomero-nasale' of de Vries and 'Nebenbulbus' of v. Gudden, Kölliker and others. "Am oralen Ende der Hemisphäre liegt medial das Ganglion terminale (Ganglion nasale, Ganglion vomero-nasale, Nebenbulbus)." From this it would appear that he considers the ganglion terminale as the primary center for the vomero-nasal nerve and he has been so understood by McCotter ('12, pp. 302, 316). This is, however, wholly inconsistent with Döllken's clear description of *roots* from the ganglion terminale to the paraterminal body and other regions of the hemisphere, and with his comparison of the cells of the ganglion terminale with those of spinal and cerebral ganglia (p. 23). On the other hand, it appears very probable to the writer that Döllken has failed to recognize the clear distinction which exists between the ganglion terminale and the cells lying among the olfactory nerve fibers ('olfactory ganglion') which later produce neurilemma cells. Comparison of his figures 1 and 2 with the conditions in the pig (figs. 1 and 3 of this paper) suggests that Döllken has in view in these stages of the mouse chiefly or solely olfactory fibers entering the formatio bulbaris and that the ganglion terminale of these figures is only the 'olfactory ganglion' of older authors. The apparent reduction in the number of ganglion cells in the course of the nerve in later mouse embryos (p. 17) would be

accounted for on the supposition that the cells seen in early stages had developed into neurilemma cells. In Döllken's figures 4 and 22 the nervus terminalis is distinct from the olfactory nerve and the root *c* of these figures corresponds to the only root seen by the writer. The fibers of Döllken's root *a* (fig. 3) appear in my preparations to belong to the olfactory nerve.

Döllken clearly confirms the work of earlier authors both as to fibers arising from the cells of the vomero-nasal epithelium and as to free nerve endings in this organ. He says:

Im Ruysch'schen Gang (Jakobson'schen Organ) und zwar nie an der Schleimhautoberfläche finden sich spindelförmige Zellen mit einer Fibrillenkontur, die einen Fortsatz in die Nervenstrecke des Nervus terminalis senden (Taf. IV, Fig. 16). Ausserdem laufen glatte Fasern vom Nerven bis zur Oberfläche der Schleimhaut, ohne in Verbindung mit einer Zelle des Sinnesorgans zu treten. Diese Fasern sind auch von v. Lenhossék u. A. gesehen und beschrieben worden. Sie kommen in der ganzen Riechschleimhaut vor. Manche Autoren haben die Vermutung geäussert, es könne sich um Trigeminafasern handeln. Für die entsprechenden frühen Fasern des Ruysch'schen Ganges glaube ich diese Annahme ausschliessen zu können. Sie stammen von Ganglienzellen des Terminalnerven.

From this it is clear that two kinds of fibers are present in the nervus vomero-nasalis of de Vries and nervus terminalis of Döllken, some arising from true olfactory sense cells in the vomero-nasal organ as described by v. Brunn ('92), v. Lenhossek ('92) and Read ('08), and others arising from the cells of the ganglion terminale.

The vomero-nasal nerve of mammals is not the homologue of the nervus terminalis of fishes. The two nerves exist side by side in mammalian and reptilian embryos. The vomero-nasal nerve arises from cells in the nasal mucosa indistinguishable from typical olfactory sense cells, while the nervus terminalis arises from bipolar ganglion cells situated on the course of the nerve and resembling cerebro-spinal ganglion cells. The vomero-nasal nerve enters the bulbus olfactorius where it has a special part of the formatio olfactoria as its center (the so-called accessory bulb or the formatio vomero-nasalis). The nervus terminalis enters the hemisphere caudal to the bulb in entirely different relations. The nervus terminalis in mammalian embryos is

clearly a ganglionated nerve connected with the neuroporic region of the forebrain and supplying free nerve endings to the vomero-nasal organ and probably to a variable part of the nasal sac. The olfactory fibers arising in the vomero-nasal organ run in the same bundles with the *nervus terminalis*, but enter the *bulbus olfactorius*.

The existence of this nerve in adult fishes and amphibians and in embryonic reptiles and mammals warrants a careful search for it in adult reptiles and mammals. Already McCotter ('12) has found strands connected with the vomero-nasal nerve in the adult dog which enter the brain in the proper position for the *nervus terminalis*. Further studies may show this nerve present in adult turtles and snakes and in various mammals. It is probable that this region of the adult human brain has never been studied in any way that is favorable to the discovery of this nerve if it should be present.

Discussion of either the morphological or physiological significance of the *nervus terminalis* would not be profitable at this time. It is clear that there exists in the most anterior vertebrate segment a receptive nerve in addition to the olfactory. The olfactory and the *nervus terminalis* may be regarded as two components of a segmental nerve, analogous to the gustatory and general cutaneous components which exist together in the VII or the IX cranial nerves in some fishes. That the olfactory nerve and *nervus terminalis* have come to have separate roots and very widely differentiated centers is no bar to this view. For the two components in the VII or IX, or in any nerve, have differentiated centers and in many cases the fibers of one component in a cranial nerve become segregated at their entrance into the brain so as to form one or more pure rootlets. It is possible that the *nervus terminalis* contains one or more other components still. Brookover's work on *Amia* seems to show that this nerve has a relation with the head sympathetic and that the larger part of the cells in its course are sympathetic cells concerned probably in vaso-motor functions. About forty fibers constitute the forebrain root of the ganglion terminale. These Brookover would regard as preganglionic sympathetic fibers. This interpretation would not be tenable if these fibers are the axones of

cells in the ganglion as he implies in his conclusions (p. 114). If they are axones of peripheral ganglion cells, they are best regarded as receptive fibers, as the writer has considered them in other fishes. That some of the cells derived from the olfactory placode along with the ganglion terminale should develop into sympathetic ganglion cells is no more than might be expected in view of the origin of sympathetic ganglion cells from the spinal ganglia. Whether any sympathetic roots remain connected with the telencephalon as a component of the nervus terminalis is still uncertain.

The continuation of some of the fibers of this nerve in urodeles to the hypothalamus and probably to the interpeduncular region of the mesencephalon as described by McKibben is very remarkable. Taken together with the apparent absence of a ganglion terminale this suggests the hypothesis that these may be efferent (sympathetic) fibers such as Brookover supposed to be present in *Amia*. Upon this point the writer would offer the observation that in a 35 mm. larva of *Amblystoma punctatum* collections of ganglion cells are found on two of the three branches of the olfactory nerve which supply the vomero-nasal organ. In view of the discussion over the vomero-nasal organ of amphibians it should be said that the development of the organ has been traced in *Amblystoma*. It arises as a medial diverticulum from the ventral part of the thick olfactory epithelium of the nasal sac a short time before the choanae are open into the mouth. Later a rotation of the nasal sac and the vomero-nasal organ takes place, such that this organ comes to hold the relations of a lateral diverticulum in the 35 mm. larva. The diverticulum very early gives rise to branched tubular glands which extend medially and lie ventro-medial to the nasal sac. The nerve supply here described is further evidence that this diverticulum is the homologue of the vomero-nasal organ of reptiles and mammals, as held by Burekhardt, Seydel, Hinsberg and others. (For the literature, see Peter '02). The small ganglia mentioned above lie near the vomero-nasal organ and correspond to the ganglia seen near the ends of the branches of the nervus terminalis in turtle embryos. As described by McKibben the nervus terminalis is imbedded in the olfactory nerve and its peripheral course was not seen. The

ganglia on vomero-nasal branches of the olfactory nerve in *Amblystoma* serve to identify the *nervus terminalis* peripherally, and show both that the nerve is ganglionated in urodeles and that it has the same distribution as in reptiles and mammals.

The evidence at present in hand seems to establish beyond doubt the presence in all vertebrates of a receptive component in the *nervus terminalis* supplying ectodermal territory. This component is derived either from the terminal part of the neural crest (Johnston '09 b, Belogolowy '12) or from the olfactory placode (Brookover '10). The nerve is distributed to the nasal mucosa or to a specialized part of it, the vomero-nasal organ. What is needed now is definite knowledge of the central connections and experimental evidence as to its function.

One further suggestion, although very vague in its present form, may be hazarded here. The close association of the *nervus terminalis* with the vomero-nasal organ in amphibians, reptiles and mammals suggests that the influence of this nerve in the fish-like ancestors of these forms may have been an important factor in the differentiation of the vomero-nasal organ. Also it may be supposed that the distribution of the *nervus terminalis* in fishes would give some indication as to the portion of the nasal sac from which the vomero-nasal organ has been derived.

SUMMARY

In embryos of the turtle, pig, sheep and man there is found a true *nervus terminalis* consisting of a ganglionated nerve whose root enters the telencephalon caudal to the *bulbus olfactorius*.

This nerve exists in addition to the *nervus vomero-nasalis* in mammals, and the *nervus terminalis* is distributed chiefly to the vomero-nasal organ. The olfactory fibers arising from the vomero-nasal organ separate from the *nervus terminalis* to enter the olfactory bulb.

The nerve root enters the brain in the line of separation—*zona limitans*—between the hippocampus above and the precommissural body and *tuberculum olfactorium* below.

In *Amblystoma* the *nervus terminalis* is ganglionated and supplies the vomero-nasal organ as in reptiles and mammals.

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ABBREVIATIONS

<i>b.o.</i> , bulbus olfactorius	<i>n.olf.v.</i> , nervus olfactorius, ventral root
<i>c.</i> , choana	<i>n.</i> , narial opening
<i>c.a.</i> , commissura anterior	<i>n.sac</i> , nasal sac
<i>f.i.</i> , foramen interventriculare	<i>n.t.</i> , nervus terminalis
<i>f.o.d.</i> , formatio olfactoria, dorsal portion	<i>olf.ep.</i> , olfactory epithelium
<i>f.o.v.</i> , formatio olfactoria, ventral portion	<i>pal.</i> , palate
<i>g.t.</i> , ganglion terminale	<i>p.h.</i> , primordium hippocampi
<i>hem.</i> , hemisphere	<i>r.n.</i> , recessus neuroporicus
<i>m.</i> , margin of lamina supraneuroporica	<i>r.p.</i> , recessus praeopticus
<i>med.div.</i> , medial diverticulum of nasal sac	<i>s.</i> , corpus striatum
<i>n.o.a.</i> , nucleus olfactorius anterior	<i>t.</i> , tectum mesencephali
<i>n.olf.</i> , nervus olfactorius	<i>t.c.</i> , tela chorioidea
<i>n.olf.d.</i> , nervus olfactorius, dorsal root	<i>t.o.</i> , tuberculum olfactorium
	<i>v.l.</i> , lateral ventricle
	<i>v-n.o.</i> , vomero-nasal organ
	<i>v.tr.</i> , velum transversum

Fig. 1 Fig, 22 mm. Model of rostral part of right hemisphere, seen from the medial surface. The bulbus olfactorius is not clearly delimited by grooves but the root of the olfactory nerve shows its position. The ganglion terminale accompanies the root and four branches of the nervus terminalis which runs obliquely across the roots of the olfactory nerve. The point *r.p.* in the model is just above and rostral to the preoptic recess.

Fig. 2 Fig, 19 mm. Sagittal section through the rostral part of the left hemisphere. The spindle-shaped ganglion terminale is closely related to a broad collection of lightly staining cells which follows the contour of the bulbus olfactorius. These latter cells belong to the neurilemma cells of the olfactory nerve. Note that the root accompanied by flattened cells is directed toward the neuroporic recess.



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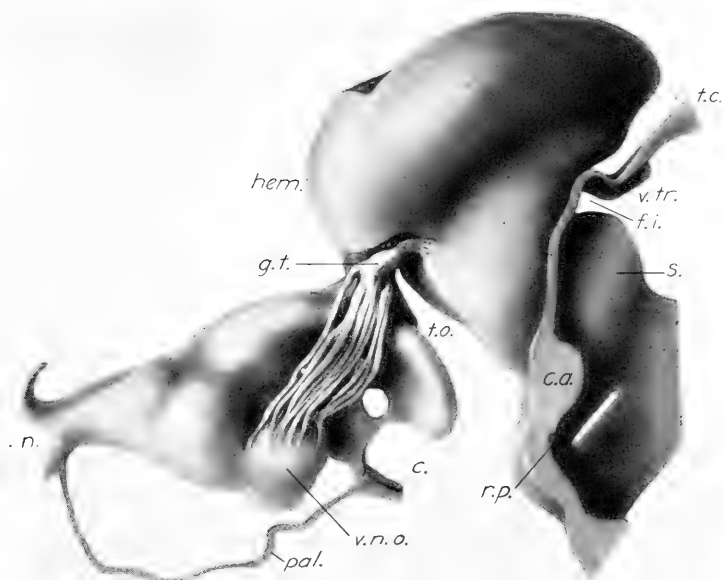


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Fig. 3 Pig, 15 mm. Model of part of the right hemisphere and nasal sac, seen from the medial surface. The model includes the optic stalk, whose slit-shaped cavity is seen, and the medial wall and frontal pole of the hemisphere. The groove in the hemisphere is the fissura prima of His. The root of the nervus terminalis is directed backward in the paraterminal region. The olfactory nerve forms a dark background for the ganglion terminale in the drawing. It is at this point that olfactory fibers coming from the vomero-nasal organ separate from the nervus terminalis fibers and enter the olfactory bulb in the olfactory nerve roots. The two kinds of fibers run together in the bundles which go to the vomero-nasal organ.

Fig. 4 Human embryo of 31 mm. (Huber collection XLVII). Model of rostral portion of right hemisphere showing the relations of the nervus terminalis. The ganglion terminale is distinct from the root of the olfactory nerve but distally the nervus terminalis fibers mingle with olfactory nerve fibers.



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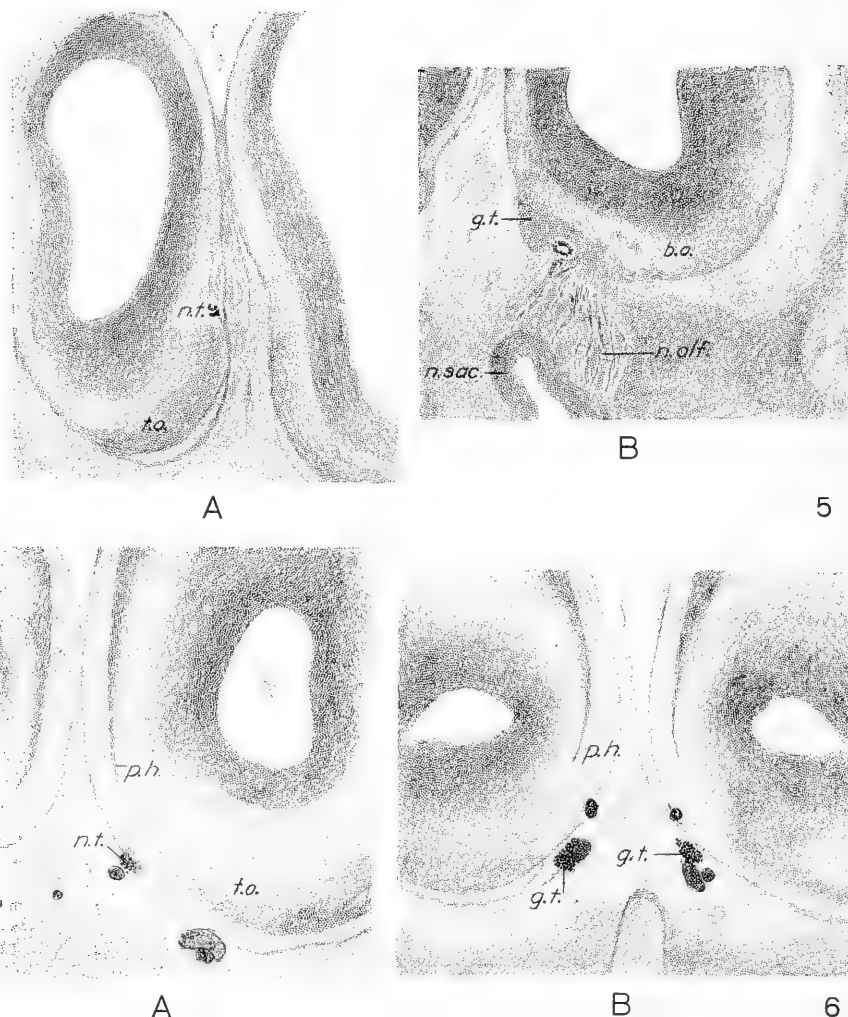


Fig. 5 Pig, 16 mm. Two transverse sections of the rostral part of the hemispheres. In *A* the root of the nervus terminalis is seen as a small collection of cells lying within the brain beneath the fissura prima and above the tuberculum olfactorium. In *B* the ganglion terminale is shown where it comes in contact with the root of the olfactory nerve. The ganglion cells are separated from the neurilemma cells of the olfactory nerve by a small blood vessel. The ganglion terminale is unusually large in this specimen.

Fig. 6 Pig, 53 mm. Two transverse sections through the hemispheres showing the root and ganglion of the nervus terminalis. Note that the root enters the brain between the primordium hippocampi and the tuberculum olfactorium.

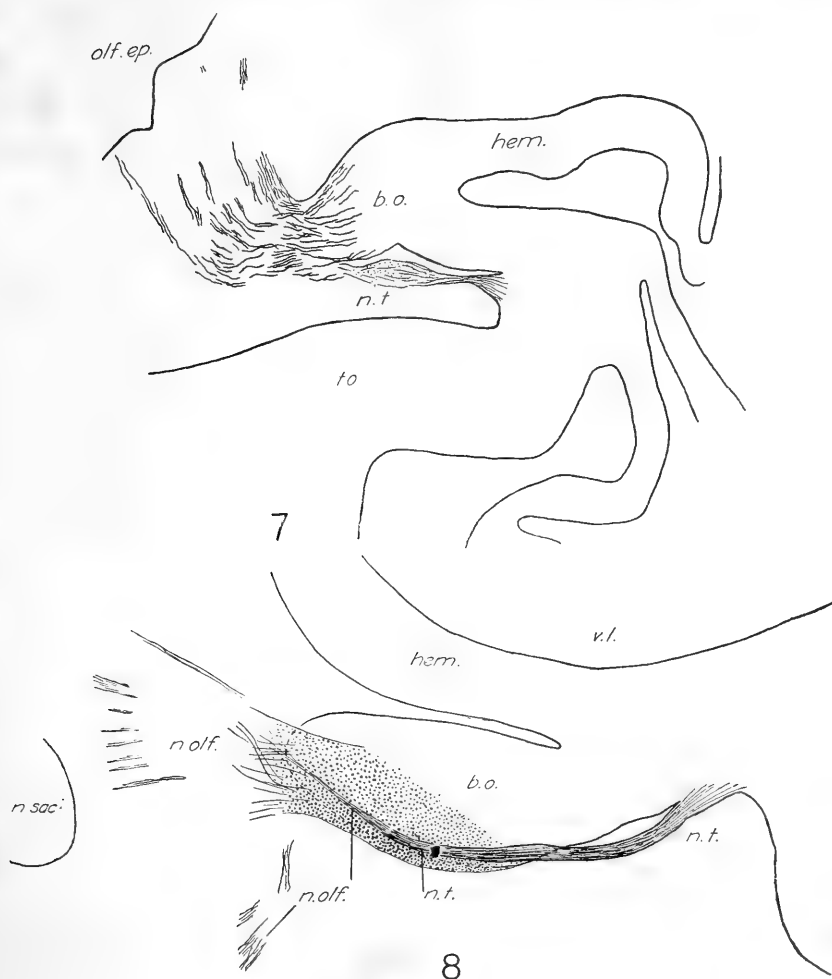


Fig. 7 Human embryo of about 15 mm. (Huber collection xxxiii). Sagittal section through the root and ganglion of the nervus terminalis. Peripherally the nerve is lost in the olfactory bundles, but its ganglion is distinct and the root enters the brain in the characteristic position.

Fig. 8 Human embryo of 47 mm. (Huber collection ixl). A projection upon one plane of the course of the nervus terminalis from its root to the point where it mingles with the olfactory bundles. The area coarsely stippled indicates the position occupied by the mass of neurilemma cells among the root bundles of the olfactory nerve. The nervus terminalis runs over the medial surface of this mass and joins olfactory bundles running in the same general direction. Only a few olfactory fibers are sketched.

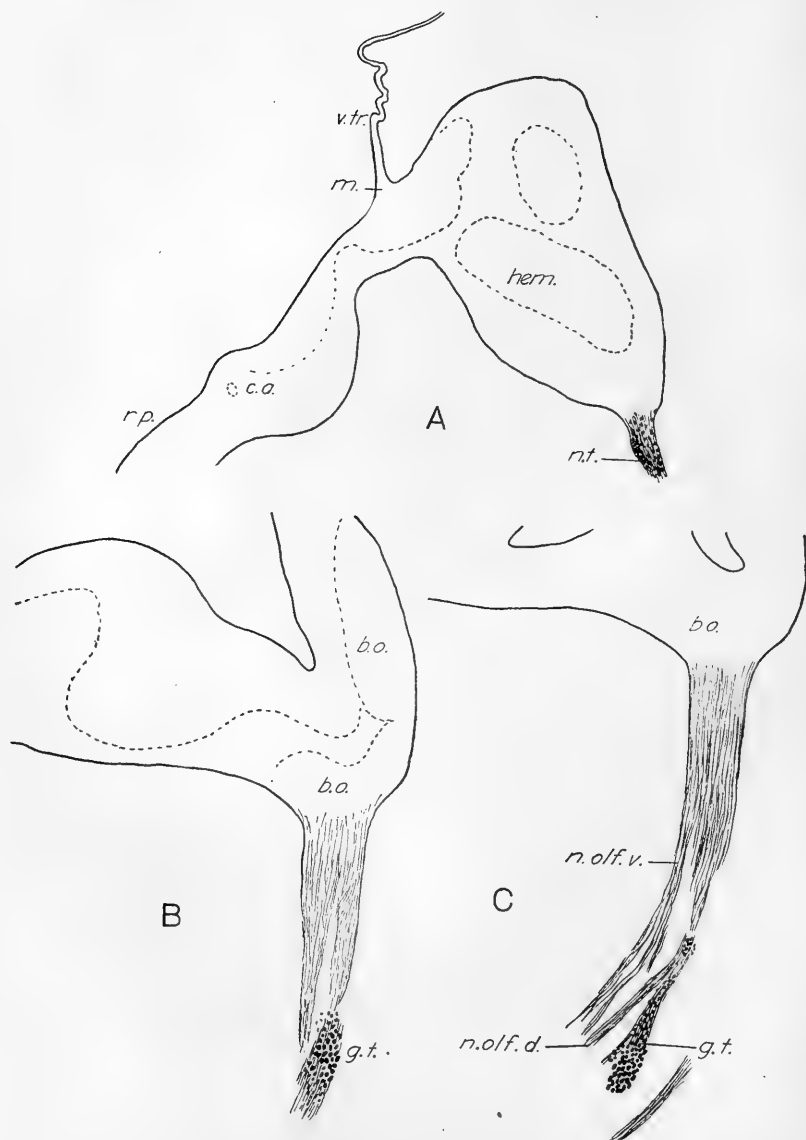


Fig. 9 *Emys lutaria*, 10 mm., three parasagittal sections. In A the nervus terminalis enters the rostral end of the hemisphere on its medial surface. It contains some ganglion cells. In B the olfactory bulb and ventral part of olfactory nerve are shown. The section passes through a ganglion midway of the length of the nervus terminalis. In C a peripheral ganglion is cut at the point where the nervus terminalis separates from the dorsal division of the olfactory nerve to enter the wall of the medial diverticulum (vomero-nasal organ).

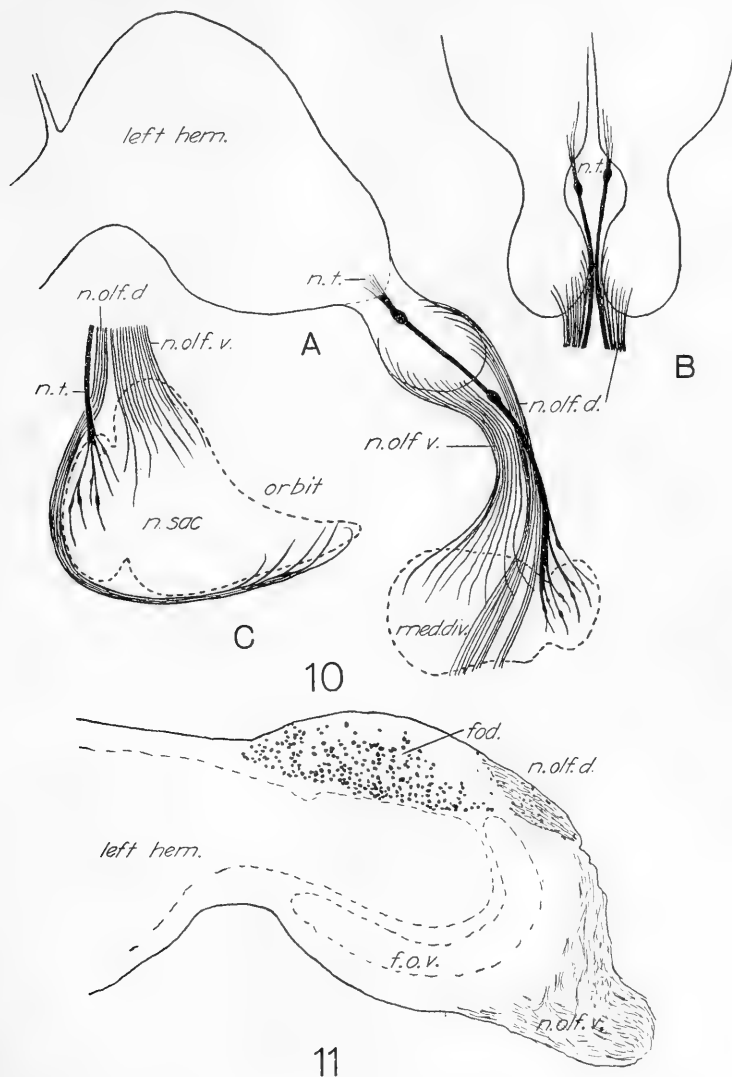


Fig. 10 *Emys lutaria*, 20 mm. Three diagrams to illustrate the course of the olfactory nerve and nervus terminalis. In A the nerves are projected on a parasagittal plane, seen from the medial direction, B, a projection on the horizontal plane, ventral part of olfactory nerve omitted, C, a diagrammatic transverse section of the nasal sac. That part of the nasal sac to which the nervus terminalis is distributed is believed to be the vomero-nasal organ.

Fig. 11 *Emys lutaria*, 20 mm. Parasagittal section showing the dorsal root of the olfactory nerve and the division of the formatio olfactoria into dorsal and ventral portions.

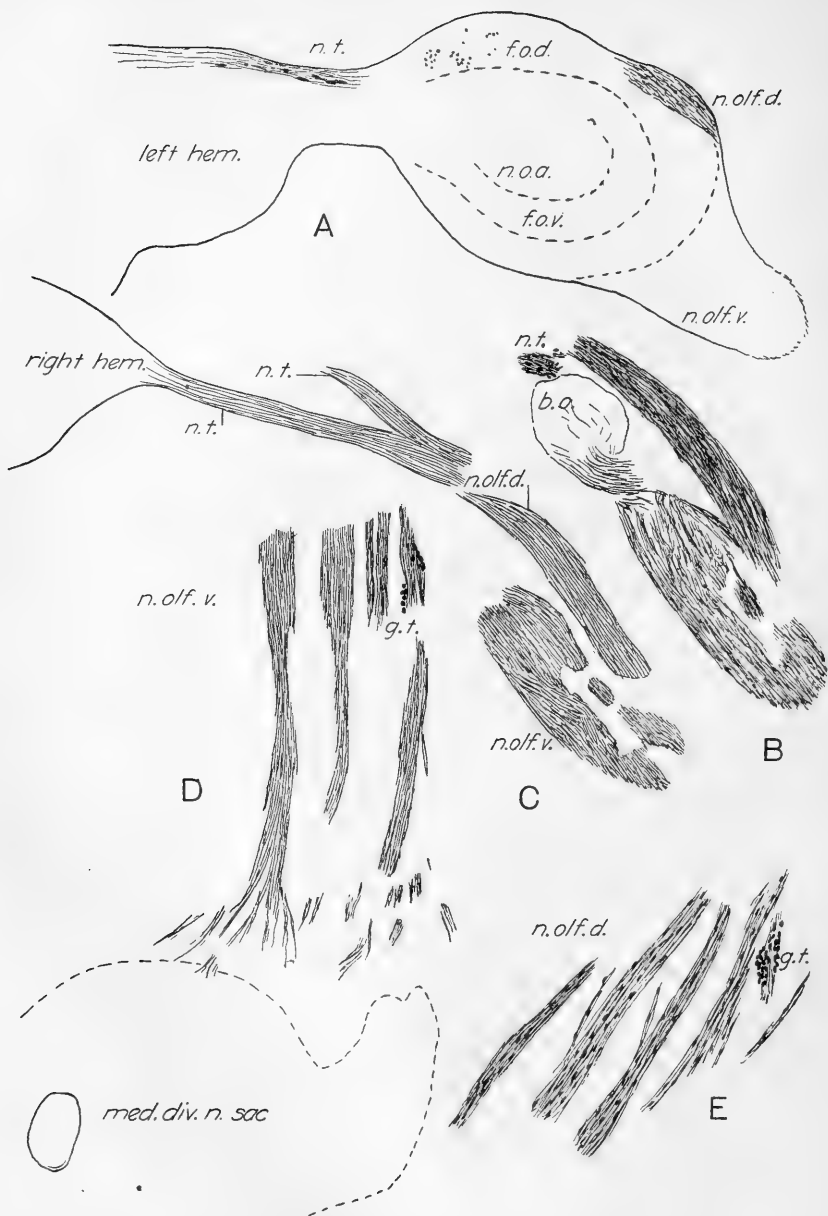


Fig. 12 Five sections from the same series as figure 11. In *A* is shown the root of the nervus terminalis entering the hemisphere behind the olfactory bulb. In *B* the nerve is almost in contact with the tip of the olfactory bulb and runs close to the dorsal olfactory root. In *C* the nervus terminalis touches its fellow (compare figure 10 *B*). In *D* the section cuts an isolated ganglion on the course of the nervus terminalis. The ventral olfactory is spreading out to its endings. In *E* the dorsal olfactory courses ventrad over the surface of the medial diverticulum. One of the small bundles of the nervus terminalis is seen with ganglion cells.

THE PRIMARY VENTRAL ROOTS AND SOMATIC MOTOR COLUMN OF AMBLYSTOMA

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TWENTY-EIGHT FIGURES

Preliminary to the publication of studies upon the development of the nervous system in relation to the development of behavior in embryos of Amblystoma this paper is intended to record and discuss briefly certain observations which, beyond their relation to the general problem of correlation of growth and function, have particular bearing upon the morphology of the nervous system. These observations have to do with the nature of the primary somatic motor column of the spinal cord and brain and the relation of the primary ventral roots to the neurones of this column. The general conception, growing out of the study of the definitive ventral horn cell, seems to have been that the neurones which form the ventral roots are a distinct type. The neurones, however, which establish the earliest contact with the cells of the myotome are found in Amblystoma to be at the same time the neurones of the motor tract in the central nervous system. The primary ventral root fiber is a collateral of a tract cell. It is this discovery with which this communication is concerned.

The species used chiefly in these studies is *A. punctatum*. Occasionally *A. opacum* has been introduced but my observations do not suggest that there is any difference between the two species on the point under consideration. The specimens were selected according to the physiological standards which an earlier paper¹ describes in detail. These standards are based on the ability of the embryo to execute somatic movements in

¹ Jour. Comp. Neur., vol. 19, no. 1.

response to tactile stimulation. In order to obtain specimens that are approaching the stage of earliest reaction to touch my method has been to apply the current from an inductorium gauged so as to stimulate a slightly older individual without injury. This test differentiates the embryos that are very near to the stage in which response to tactile stimulation becomes possible, for the somatic muscles can be stimulated by electricity in this manner for a brief period before they can be stimulated through the tactile receptors. Embryos in this phase of development represent the earliest stage taken account of in this study. Following this, in the natural order of development, are specimens of the early flexure stage (which contract only the most rostral myotomes), specimens of the 'coil-reaction' stage (which bend the trunk into a tight coil), specimens in the 'S-reaction' stage (which perform a compound flexure or sinuous movement) and specimens of the early swimming stage. The latter specimens are approximately 7 mm. long. Drawings and descriptions of the root fibers of all these stages and remarks upon the methods of fixation and preparation are given in connection with the explanation of the figures. The scope of the paper is in no sense intended to cover or take account of the literature upon the subject. This part of the work must be deferred till the publication of the general results of my studies upon correlation of growth and function.

1. CONDITIONS FOUND IN PARTICULAR CASES

1. Embryos reacting to electricity but not to touch

The conditions of the root fibers as they occur in the youngest embryos of my collection, that is to say, in embryos which react to electrical but not to tactile stimulation, are illustrated in figures 1 and 2. The plane of section in figure 1 is approximately frontal, but tipped slightly ventro-laterad on the side figured. The most ventral portion of the central canal appears in the section, although the figure reaches only about half way from the periphery of the cord to the canal. The more mesial, elongated nuclei of the figure represent the nuclear characteristics

of the more central portion of the section. Near the periphery of the cord are nuclei of very different form. They are large and approximately spherical. The indistinctly differentiated perikarya to which these nuclei belong (*VC*) are crowded with yolk spherules, some of which occupy indentations in the nuclei. In fact the nuclei seem to partially surround such spherules in an ameboid fashion. Among these peripheral cells of figure 1 are at least two (*VC*) which clearly belong to the motor column. The more rostral one has a distinctly differentiated descending process (*DP*) which runs longitudinally immediately within the external limiting membrane of the cord. There is also a suggestion of an ascending process. The absence of a clearly differentiated ascending process in the case of this neurone is explained by the appearance of the more caudal neurone (*VC*) which lacks its nucleus and descending process. Obviously the plane of section is such that a section which passes through the nucleus of a tract cell includes the descending but not the ascending process; while a section which passes a little ventrad of the nucleus, as the section does in this case, strikes through the ascending process. Accordingly the section next dorsad of this shows a typical tract cell nucleus in the position that corresponds with this neurone and not less than three others of the same class distributed more caudally in a series. The more caudal neurone of this figure, then, belongs also to the motor column, and from its ascending process arises a branch which passes through the external limiting membrane as a root fiber to the third myotome. In this section this root fiber reaches the anlage of the spinal ganglion, among the cells of which it can be recognized in the adjacent section.

Since the first post-otic myotome ordinarily has no motor root (a root has been found in at least one instance), the root represented in figure 1 (*VF*) is the second motor root and is associated with the anlage of the first spinal ganglion. The root to the second myotome in this specimen can also be identified. While the plane of section through it does not permit positive conclusions regarding its mode of origin the root fiber certainly either

passes out of the tract from caudad as an axone or arises as a collateral from the ascending process of a tract cell.

Traced caudad in this embryo the fibers of the motor column can be identified as far as the fourteenth myotome. Beyond this a single fiber may be seen in occasional sections as far as the seventeenth myotome. Ventral root fibers can be recognized as far caudad as the thirteenth myotome, that is, there are at least twelve pairs of ventral roots in this specimen.

The root fiber indicated in figure 2 (*VF*) goes to the fifth myotome of an embryo of the same physiological age as that from which figure 1 was taken. Although the cell body of the neurone from which the root fiber arises does not appear in this section, the tract fiber has the appearance of a descending process (*DP*). The slight outward bend of the fiber at the point of origin of the collateral which passes into the nerve root is characteristic of this early stage of development.

Slightly rostrad of the root fiber in this section is a characteristic nucleus of the motor column and its perikaryon can be indistinctly seen (*VC*). The peripheral end of this cell spreads out against the external limiting membrane and extends rostrad and caudad in ascending and descending processes.

Root fibers occur in this embryo as far caudad as the eighth myotome, and uncertain suggestions of roots appear at the levels of the ninth and tenth myotomes. The fibers of the latero-ventral tract may be traced caudad as far as the twelfth myotome.

2. Embryos of the early flexure stage

Conditions found in embryos very soon after they first respond to tactile stimulation are represented in figures 3 to 9, inclusive.

The section from which figure 3 is taken is in an approximately frontal plane, tipped slightly ventro-caudad and slightly dorso-laterad on the side figured, so as to leave a point of the latero-ventral portion of the spinal cord between the notochord and the eighth myotome. The descending process of a neurone (*DP*), the nucleus of which is not included in this section, branches into two divisions close to the external limiting membrane. One

division, apparently the larger in this case, passes out of the cord as a root fiber (*VF*) and reaches almost to the anlage of the spinal ganglion (*SG*). The other division continues caudad in the latero-ventral tract. Descending processes of other cells enter this root, as shown in the figure, without giving any evidence of bifurcation beneath the limiting membrane, but there is nothing in the preparation that would render such a branching improbable. In fact, slightly caudad of the root are clearly differentiated fibers of the tract which may well be the descending processes of these same neurones.

Figure 4 is drawn from the opposite side of the same embryo and the section is therefore tipped slightly latero-ventrad. It shows a root fiber (*VF*) arising clearly as a collateral of a tract fiber and passing through the anlage of the spinal ganglion to the muscle cell (*MC*). Its ending upon this muscle cell can not be regarded as clearly demonstrable in this section but the fiber expands slightly upon the surface of the cell and apparently ends there. Just caudad of the region of this root collateral is the basal portion of another fiber which almost certainly has the same relation to the neurone of the tract. The origin of these root fibers from the tract opposite the middle of the myotome and their application to the muscle cell directly opposite a large nucleus against which impinges a conspicuous mass of pigment (*P*) is characteristic of these early neuro-muscular relations. The outward thrust of the axone of the tract at the point of origin of the root collateral is also characteristic, as shown in figure 4.

The form of the neurones of the motor column is illustrated in figure 5 (*VC*). The neurone of the figure occurs at the level of the thirteenth myotome, some muscle cells of which (*MC*) are shown in the figure. The position and general relations of this neurone in the cord are shown in figure 6 (*VC*), from which the plane of section of figure 5 can be readily interpreted. Although the portion of the cell visible in this section is only about half the length of the adjacent myotome, the entire cell probably exceeds the myotome in length. Another neurone of this column, located at the level of the twenty-first and twenty-second myotomes (fig. 8, *VC,M*) is shown in figure 7, and its general rela-

tions in the cord are shown in figure 8. In this case both the descending and ascending processes can be readily followed for some distance, and the ascending process, presumably the dendrite, is branched. There appears, also, to be a small branch off of the descending process (*DP*) near its base, while the main process ends abruptly and in such relations as to indicate that it actually extends a considerable distance beyond the limits of this section. The tangential section of the nucleus of this neurone, as shown in the figure, is in keeping with the form of the tract cell as demonstrated in other planes of section, which show the neurones with their perikarya shunted off to one side of the main fibrillar axis. The distribution of the yolk spherules in accordance with the fibrillar structure around the nucleus, as shown in this figure, is typical.

There is no evidence of ventral roots in this embryo at the level of the cell of figure 7; and the general appearance of the myotomes of that level as shown in figure 8 would not lead one to expect ventral roots at this level. However, a study of the two adjacent myotomes throughout their entire extent in the serial sections reveals a few cells in their most ventro-mesial portion that might have muscular function. Comparative studies of embryos, on the other hand, lead me to believe that at this stage the ventral root system has not extended so far caudad, and that this tract cell, therefore, lies considerably caudad of any ventral root.

Another case of the origin of a ventral root fiber as a collateral from a tract neurone in an embryo of this age is shown in figure 9. Here the plane of section is in general vertical, but the embryo is twisted in such a way that at the level of this root the section is very nearly in the frontal plane, being tipped considerably latero-ventrad on the side figured. A careful study of this root fiber (*VF*) by varying the focal plane gives one the impression that the fiber from which the collateral arises is a descending process of a tract neurone, and the angles subtended by the collateral and the axone corroborate this interpretation. Here again is seen the characteristic outward thrust of the axone at the point of origin of the root collateral.

3. Embryos of the 'coil-reaction' stage

The origin of root fibers at various levels in embryos of the 'coil-reaction' stage is shown in figures 10 to 15, inclusive.

The root of figure 10 goes to the seventh myotome. The figure is drawn from an approximately frontal section, tipped slightly dorso-laterad on the side figured. There are several fibers in the root, towards the base of which the fibers of the tract arch outward. From one of these a root fiber (*VF*) clearly arises as a collateral; in the case of another, not clearly shown in the figure, the same mode of origin is practically certain. The opposite root to the seventh myotome is drawn in figures 12 and 13 (*VF*). In this case the root process of the cell extends directly outward through the external limiting membrane, immediately beneath which ascending and descending processes are given off into the tract. Figure 12 shows the focal plane in which the connection of this neurone with the nerve root is clearest, while figure 13 shows the appearance in the focal plane which demonstrates the relation of the neurone to the ventro-lateral tract.

The root to the eleventh myotome of the same specimen is shown in figure 11, where the collateral (*VF*) arises from the descending process of a neurone of the motor column. While the cell body can not be distinctly made out in this section the general form of the process leaves no doubt as to its nature. Again, in this section, the arching outward of the axone at the origin of the root collateral is conspicuous.

Far caudad of demonstrable ventral roots in this specimen may be recognized the longitudinally oriented neurones of the motor column. One of these neurones, located at the level of the thirty-third myotome, is drawn in figure 14 (*VC*). This neurone lies in the extreme ventro-lateral region of the spinal cord. Its descending process (*DP*) is more clearly seen in the section than is its ascending process, though this inequality may be due to the plane of section. The yolk spherules in the basal portion of the descending process conform to the polarization of the cell. At this level and in this plane of section the medullary tube is constituted of a single layer of short epithelial cells, excepting as the

neurones of the ventral column and the neurones of the dorsal column, the giant ganglion cells, are differentiated in the most peripheral part.

The structure of the myotome opposite this root is drawn in figure 15. Careful examination of this myotome and others of the immediate region gives no evidence of their having differentiated muscle cells in any part. This fact, together with the fact that in specimens that are physiologically much more advanced in development than that from which this figure was taken, root fibers can be demonstrated only as far caudad as the twenty-seventh myotome, leaves no doubt that, in this level of the cord, the ventro-lateral tract neurones become differentiated and oriented considerably in advance of the development of ventral roots.

4. Embryos of the 'S-reaction' stage

A group of neurones of the motor column is shown in figure 16. These neurones are found at the level of the twenty-second myotome of an embryo of the 'S-reaction' stage, that is, an embryo that can perform the sinuous, double flexure, but can not perform this movement in series so as to effect locomotion. The plane of section here is approximately frontal, but tipped slightly dorso-laterad on the side figured and also slightly dorso-rostrad. The figure, accordingly, shows the ascending processes (*AP*) to better advantage than it does the descending processes (*DP*), though considerable portions of the latter are perceptible in one or two of the neurones. It is possible here to demonstrate the ascending process (*AP*), presumably the dendrite, of one of these tract neurones for a distance of one-half the length of the myotome. The descending process, my general observations lead me to believe, is ordinarily considerably longer than the ascending process. If this is true, the entire length of one of the tract neurones at this level and in this stage of development must be considerably greater than the length of the myotome.

The relation of the neurones of the motor column to those of other parts of the cord at this age is shown in figure 17. This figure is taken from a section approximately parallel to the sagit-

tal plane. In the ventral part is seen the ventro-lateral tract (*VT*) into which the broad processes of the neurones of the motor column (*VC*) project. In the dorsal region appear the perikarya of the giant ganglion cells which form the dorsal sensory column. The nuclei in this figure are all drawn with the aid of the camera lucida. A general idea, therefore, can be formed from this drawing of the relative size and similarity in arrangement of these two types of neurones. Here the nuclei of the motor column are distinctly smaller than those of the giant ganglion cells, and my observations lead me to believe that they are generally so. It is noteworthy also that while there is a sharp differentiation of the giant ganglion cells from all others around them, there is more or less of a gradual transition from the typical, large, round nucleus of the motor column and the elongated nuclei of the ependyma. A corresponding gradation in nuclei is also seen in certain staining reactions. Such difference in differentiation is obviously correlated with the difference there is between these motor and sensory cells in the further development of the animal. The giant ganglion cells are transitory and do not become permanently worked into the definite organization of the spinal cord, while the motor cells are part of a developing system which elaborates with the development of the animal. The motor system, therefore, is here a developing system whereas the dorsal ganglion cell system has reached its full development so far as cell differentiation is concerned.

5. Embryos of the early swimming stage

The general relation of the motor and sensory neurones of the cord in an older specimen is shown in figures 18 and 19. Figure 19 is drawn through the cord at the level of the ninth myotome, while figure 18 is taken from the level of the eighteenth myotome. The ascending process of the giant ganglion cells (*DC*) may be seen here projecting slightly ventrad toward the dorso-lateral tract (*DT*). The neurones of the motor column (*VC*) lie mesially or slightly dorsally of the ventro-lateral tract (*VT*). Their peripheral ends project into the tract and bifurcate into ascend-

ing and descending processes, relations that are clearly demonstrated in other planes of section. For instance, in figure 20, taken from an embryo of the early swimming stage, is drawn a neurone of the motor column at the level of the thirtieth myotome. The plane of section here is approximately frontal, but tipped slightly ventro-caudad and dorso-laterad on the side figured. The entire section of the thirtieth myotome is also shown in the figure, and the section through the skin as well. The broad body of the cell, with its characteristic enclosure of yolk spherules, projects laterad against the external limiting membrane and extends caudad in a slender descending process (*DP*). The basal portion of its ascending process is also perceptible. The position of this neurone of the motor column is considerably caudad of any demonstrable nerve roots.

At the level of the twelfth myotome of an animal of the same degree of development the mode of origin of both the ventral roots is perfectly clear and the conditions are reproduced in figures 24 to 26, inclusive. In figure 24 a number of fibers sweep from cephalad out of the latero-ventral tract (*VT*) into the ventral root which passes directly into the anlage of the spinal ganglion (*SG*). One of these fibers (*DP*), isolated from the others, shows the branched condition and the collateral (*VF*) to the root. The characteristic loop made by the fibers of the tract at the origin of the root is better shown in the root of the opposite side, as drawn in figure 25. In the section of figure 24 the caudal arm of the loop is cut away excepting for a few scattering fibers. In figure 25 both the rostral and caudal arms of the loop are clearly seen, and one of the fibers of the loop shows the branched condition distinctly. This fiber is drawn alone in figure 26, where the root fiber (*VF*) is seen as a collateral of a fiber which continues caudad (*DP*) in the latero-ventral tract. A neurofibril is shown going into each of these divisions of the fiber. In figure 24 there is apparently a branching of a single neurofibril with one branch going into each of the processes, but in this, and in other similar cases, two fibrils may be in very close apposition with one another so that their separation in the given plane of section appears like branching. Such appearance occurs also in figures 21, 22 and 27.

Figure 27 is taken from the level of the tenth myotome of the same specimen as that of figure 24. Here, again, the loop of the tract fibers is perceptible, particularly in the case of the fiber from which the collateral (*VF*) arises. This root fiber arises from the rostral arm of the loop, while there can be no doubt that the caudal arm of the loop extends on caudad into the tract. The appearance of the fiber at the branching would indicate that the fiber from which this root collateral arises is a descending process of a tract neurone. This is the case also in figure 22, which is drawn from the level of the ninth myotome of an embryo of the same stage of development. The arching outward of the tract fibers at the origin of the root is apparent here also, and particularly is this true in figure 23, which is drawn from a different focal plane of the same field. The disappearance of the more mesial fibers of the tract in the middle of the loop as shown in figure 23 is caused by their passing out of the plane of section as they deflect from their regular longitudinal course in the tract. This deflection in the fibers of figure 23 is directly towards the origin of the root in figure 22. In the case of figure 21, which is drawn from the root of the eighth myotome of the same embryo as the last, the root collateral has the appearance of arising from an ascending process. The plane of section, however, is here very oblique and the exact relations difficult of interpretation. The root fiber arises from a tract fiber which appears to be the axone of the cell marked *VC*, and the direction of this main process is almost if not quite laterad. The relations here are probably the same as those demonstrated by a different method in figures 12 and 13.

The most advanced stage of development of the ventral root system under consideration is represented in figure 28, which is drawn from the first ventral root of an embryo of the early swimming stage. In this case a densely impregnated fiber which lies deeply embedded in the ventro-lateral tract arches outward slightly towards the root of the first spinal nerve and gives off a collateral (*VF*) which passes directly laterad among the fibers of the tract and enters the nerve root.

2. DISCUSSION OF THE RELATIONS IN GENERAL

From the foregoing particular descriptions it is obvious that neurones of the somatic motor column become well differentiated and typically oriented in the spinal cord before the ventral roots appear in the corresponding level. The earliest demonstrable root fibers arise as collaterals from these neurones. The great majority of clear cases indicate that the collaterals regularly arise from descending processes excepting in the most rostral nerves. In these nerves—the first, second and possibly the third pair—the root collaterals may arise from ascending processes.

This origin of the motor roots from descending processes of tract neurones is in harmony with the general process of integration of the nervous system, which, in these early periods of development, conducts, in the interest of locomotion, all stimuli from the skin directly to the rostral end of the muscle system, and thence caudad so as to produce a wave of contraction that progresses cephalo-caudad. Since the neurones of the descending tract are at the same time the neurones of the ventral roots their polarization would require that root fibers arise from the neurite, or descending process. In the region of the most rostral myotomes, however, the neurites seem to ascend in the motor column. While this may seem at first thought to be inconsistent with the general plan of integration of the motor system it is not so in reality, for, while it is necessary for locomotion that the wave of contraction be stimulated in cephalo-caudal progression, the simultaneous contraction of several of the most rostral myotomes of the same side would not interfere with the efficiency of the movement. Such simultaneous contraction of myotomes in the rostral region would tend to be produced by the introduction of ascending processes in the motor path to the myotomes, for, in embryos of the early flexure stage, the sensory field lies chiefly caudad, in the territory of the giant ganglion cells, and the sensory path ascends to the region of the most rostral myotomes before it can reach the motor system. This it accomplishes at the level of the most rostral myotomes and from this center the motor column can participate in the ascending conduction to

the most rostral one or two, or possibly three myotomes without interfering with the locomotor efficiency of the muscular system.

In later stages of development the more rostral ventral roots certainly receive collaterals also from descending processes of the tract. This is correlated with the introduction of the cranial sensory field through the descending trigeminal tract, the ventral commissure and the extension rostrad of the motor column.

It seems necessary, therefore, to regard the motor column at the level of the most rostral myotomes to be both descending and ascending in embryos of the early swimming stage. Just how far caudad this ascending conduction may occur in the column it is impossible to say at present. There are suggestions that it advances caudad in development *pari passu* with the extension caudad of the ventral commissure, but this is not demonstrable with the material at hand. A more critical discussion of this question must await the description of the sensory and commissural systems.

In the very early condition the axone of the tract bends outward slightly at the origin of the root collateral (figs. 2, 4, 9, 11). Later the outward deflection at this point affects numbers of fibers and a large element of the tract bends outward in an abrupt loop, from or near the tip of which the root collaterals arise (figs. 22, 23, 24, 25, 27). This arrangement suggests Johnston's figure of the dorsal roots of *Amphioxus*² where fibers of the tract deflect out in long loops into the dorsal roots. Johnston suggests that this condition is produced by the outward migration of cells from the cord along the roots. No such agency, however, can account for the condition in *Amblystoma* embryos for during the periods under consideration there is no perceptible migration of cells from the cord into or along the roots. Furthermore, to recur to the condition in *Amphioxus*, Johnston describes the visceromotor fibers as running some distance in the cord longitudinally before they go out into the root. The idea that some at least of these deflected fibers in *Amphioxus* may be motor immediately suggests itself. Those long loops into the root may

² J. B. Johnston, The cranial and spinal ganglia and the visceromotor roots in *Amphioxus*; *Biological Bulletin*, vol. 9, no. 2, fig. 4.

be concerned in the origin of root fibers in *Amphioxus* as they certainly are in *Amblystoma*.

The further development of the definitive ventral roots probably involves the differentiation of neurones within this primary somatic motor column. The primary condition consists in a slender collateral which arises from a comparatively large axone that has been oriented in the column for some time before the collateral makes its appearance (figs. 14, 20, 7, 9, 4). Neurones entering the root later progressively enlarge their collateral branch at the expense of the main process to the tract (figs. 10, 11, 12, 13). This change probably involves the differentiation of neurones in the immediate vicinity of the primary root after the collateral has established itself. As the muscular function of the myotome increases neurones arise which devote their entire neurite to the formation of the root while their dendrites have only insignificant or a very subordinate part in the longitudinal conduction. Such a mode of later differentiation is not established in detail but numerous observations render it plausible.

3. THEORETICAL CONSIDERATIONS

1. Regulation in the origin of the motor roots

There is a definite correlation of the outgrowth of the primary root collateral and differentiation within the myotome. The collateral always grows to the middle of the muscle cell and applies itself to the cell at the point opposite a large nucleus against which impinges a conspicuous mass of pigment. In the earlier condition of the mesoderm pigment is quite generally distributed as small granules throughout the cells of the myotome. As the cells differentiate into muscle cells this pigment becomes segregated into this central mass. This is evidence of some sort of a polarization of the middle of the muscle cell with reference to the ends. The motor fiber invariably grows to this central region of polarization. The exact time relations between the outgrowth of the collateral and the centralization of pigment are not yet determined but the two processes are very distinctly correlated.

It is further obvious that the differentiation of the neurones of the motor column and their orientation in the latero-ventral tract are correlated with the more general differentiation of the mesoderm into myotomes. It seems plausible, therefore, that the general and more diffuse process of differentiation within the mesoderm stimulates the differentiation of the motor neurones and their orientation and outgrowth into the tract in a longitudinal direction, while the more localized differentiation within the myotome, related directly to muscular activity, stimulates the origin of the collateral, its growth laterad through the limiting membrane of the cord and its advance to the muscle cell. This hypothesis is founded only on the general growth processes. It is susceptible to experimentation by growth of the tissues *in vitro* and it is hoped that this method may yet be applied to the details of this problem of correlative development.

2. Questions of cytomorphic and functional development

It has been noted above that ventral root fibers occur in their full relation between the spinal cord and the muscle some time before the muscles can be stimulated through the sensory field. In one embryo of this stage in development as many as twelve pairs of roots exist. It is clear, therefore, that the physiological properties of these root neurones can in no exact sense be determined through stimulation of the sensory field, for they may be actually functional for some time before they come under the influence of the sensory nerves. It is only after all the elements of a reflex arc are established that a reaction can be elicited through it. My observations upon these embryos show that all the elements in the primary and most elementary reflex arc do not become established simultaneously. Both the motor and sensory elements in this arc are extensively differentiated and in their usual relation to their end organs for some time before the development of associative neurones puts them into such relation to each other as to make reaction to stimulation possible. It would be illegitimate, therefore, to infer that any particular feature in cytomorphic development within the motor column was

definitely correlated with the development of nervous function simply because that feature arose simultaneously with the first reactions to stimulation of the sensory field. In the attempt to correlate cytomorphosis with function one should first be sure of the physiological value of the particular nerve center with which he is concerned. Certainty on this point can be established only by intensive studies of systems of neurones in their relation to each other and in relation to the development of particular bodily activity; and the observations presented here, it is hoped, may contribute something towards that end.

3. The primitive nature of the primary motor column

The discovery that the primary ventral root fibers are collaterals of neurones of a longitudinal tract places the motor system at once in the same class with the primary sensory system of the aquatic vertebrates, namely, the giant ganglion cell system of fishes and Amphibia. As is well known the primary sensory fibers in these forms are dendritic branches of central cells. This sensory system is generally conceded to be primitive and, since the primary motor system is identical with it in the general plan of organization, this motor system must also represent a primitive condition in the nervous system. While the giant ganglion cell system is known to be transitory in the life history of the individual it is impossible to say whether the primitive condition of the motor system becomes obliterated or simply obscured by the later development of the definitive ventral roots.

4. The theory of nerve components

This analysis of the primary motor column and the demonstration of its primitive nature afford new and positive conformation of the central idea of the American theory of nerve components, namely, that the primary and most fundamental divisions of the vertebrate nervous system are longitudinal and differentiated upon the basis of unity of function within each division. These divisions are regarded as somatic sensory, visceral sensory,

visceral motor and somatic motor. The latter division, in its primary and primitive condition is now found to be, not a 'pile' of discrete metameric divisions more or less indefinitely associated by means of longitudinal connectives, but a 'column' in the strict sense of a continuous, functional unit. The metameric arrangement of the motor roots, as suggested above, is probably determined by regulation and accordingly is not paralleled by a metameric arrangement of neurones within the column. The neuromeric organization of the spinal cord, therefore, in so far as it exists, is a secondary acquisition.

ABBREVIATIONS

<i>AP</i> , ascending process of a neurone	<i>MC</i> , muscle cell
<i>C</i> , notochord	<i>P</i> , pigment
<i>CC</i> , central canal of the spinal cord	<i>S</i> , spinal cord
<i>DC</i> , giant ganglion cell of the dorsal column	<i>SG</i> , anlage of the spinal ganglion
<i>DP</i> , descending process of a neurone	<i>VC</i> , cell of motor column
<i>DT</i> , the tract of the giant ganglion cells, dorso-lateral tract	<i>VF</i> , ventral root fiber
<i>M</i> , myotome	<i>VT</i> , ventro-lateral tract of the somatic motor column
	<i>Y</i> , yolk globule

All the figures have been drawn with the aid of the camera lucida. In all but figures 6 and 8, with which lower magnification was required, the lenses employed with the camera were the Zeiss Homog. oil-immersion 2 mm. objective and Compensating ocular 6. In nearly every case the compensating ocular 12 was introduced for critical study in the completion of the drawings. The numbers introduced in parenthesis in the explanation of particular figures record the serial number of the specimen in the collection, the number of the slide and section. In all figures from longitudinal sections the upper end of the drawing as it is arranged on the plate is directed rostrad in the animal.

Fig. 1 From a specimen (545, 1-6-8) which reacted to electrical but not to tactile stimulation; fixation, Van Gehuchten's fluid (alcohol-chloroform-acetic acid); stain, erythrosin and toluidin blue; sections, 5 μ , in the frontal plane.

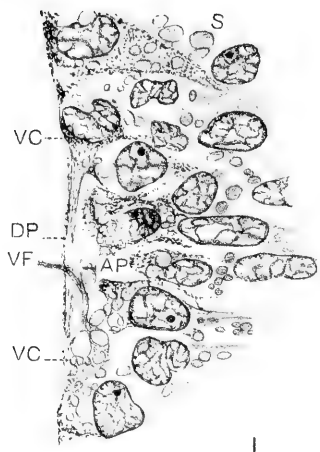
Fig. 2 From a specimen (546, 6-3-2) of the same description and treated with the same methods as the last.

Fig. 3 From a specimen (476, 1-2-20) of the early flexure stage; fixation, sublimate-acetic; staining, alum carmine in toto and Lyon's blue in 95 per cent alcohol; sections, 10 μ , in the frontal plane.

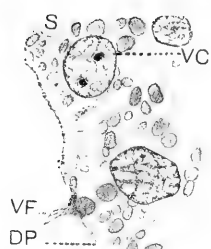
Fig. 4 From the same specimen (476, 1-2-16) as the last.

Figs. 5 to 6 From a specimen (475, 2-1-8) of the same description and treatment as the last.

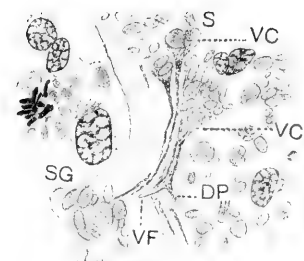
Figs. 7 to 8 From the same specimen (475, 1-3-12) as the last.



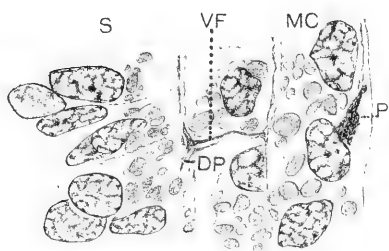
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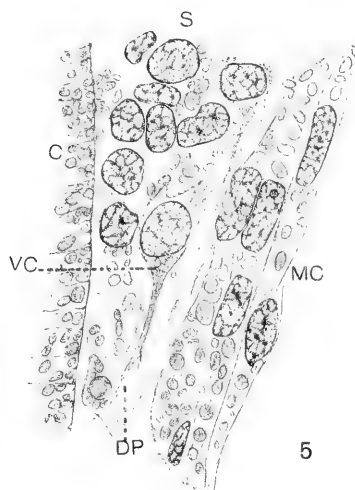
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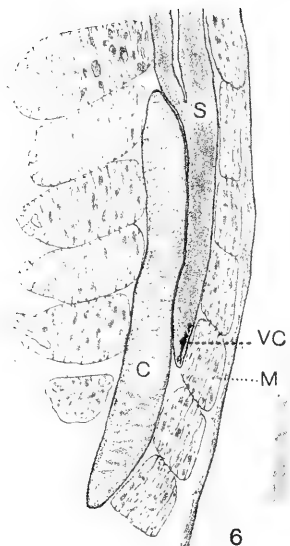
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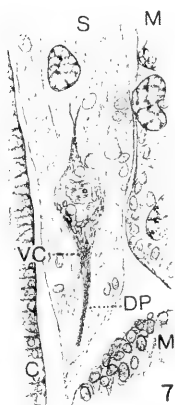
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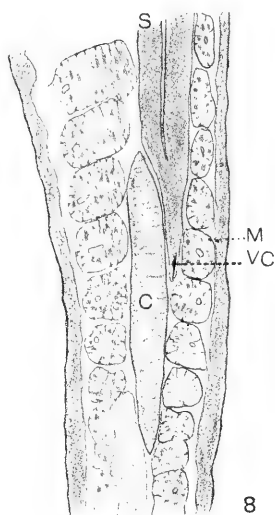
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Fig. 9 From a specimen (551, 5-2-16) of the early flexure stage; fixation, Van Gehuchten's fluid; staining, erythrosin and toluidin blue; sections, 7μ , longitudinal and in an obliquely vertical plane.

Fig. 10 From a specimen (564, 3-2-19) of the early 'coil-reaction' stage; fixation, Zenker's solution; staining, iron hematoxylin; sections, 7μ , in the frontal plane.

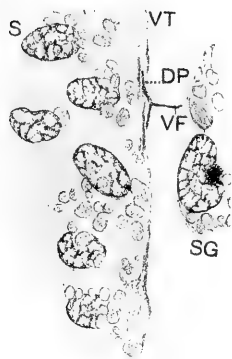
Fig. 11 From the same specimen (564, 3-2-17) as the last.

Figs. 12 to 13 From the same specimen (564, 3-2-18) as the last.

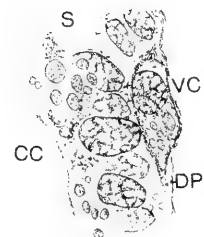
Figs. 14 to 15 From a specimen (555) of the typical 'coil-reaction' stage; fixation, Van Gehuchten's fluid, staining, erythrosin and toluidin blue; sections, 7μ , longitudinal and in an obliquely vertical plane.

Fig. 16 From a specimen (505, 1-2-15) of the 'S-reaction' stage; fixation, sublimate-acetic; staining, alum carmine in toto and Lyon's blue in 95 per cent alcohol acidulated slightly with hydrochloric acid; sections, 10μ , in the frontal plane.

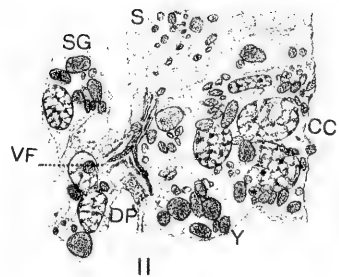
Fig. 17 From a specimen (503, 1-2-11) of the 'S-reaction' stage, treated as the last but in the sagittal plane.



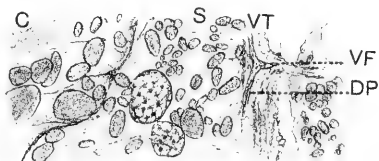
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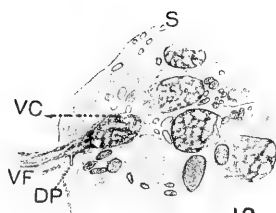
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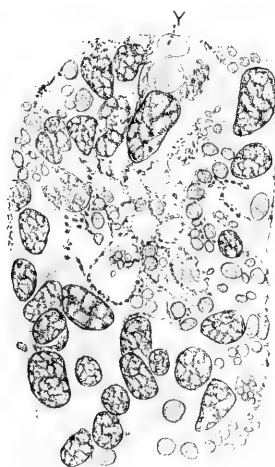
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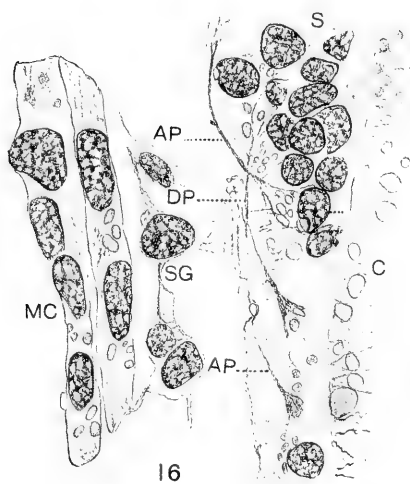
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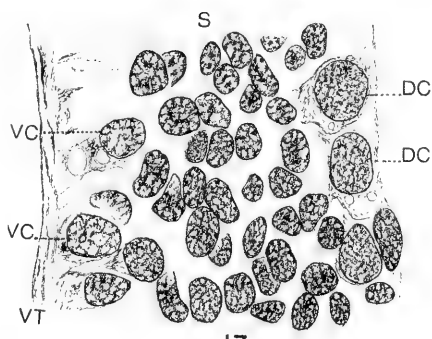
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Fig. 18 From a specimen (444, 4-4-6) of the early swimming stage; fixation, sublimate acetic; staining, Bömer's hematoxylin and orange G in 60 per cent alcohol slightly acidulated with hydrochloric acid; sections, 10 μ , transverse.

Fig. 19 From the same specimen (444, 2-2-5) as the last.

Fig. 20 From a specimen (570, 2-2-5) of the early swimming stage; fixation, Zenker's fluid; staining, iron hematoxylin; sections, 7 μ , in the frontal plane.

Fig. 21 From a specimen (412, 5-1-3) of the early swimming stage; fixation and impregnation according to Paton's modification of the nitrate of silver method; sections, 6 $\frac{2}{3}$ μ , in the frontal plane.

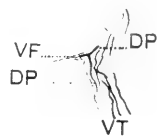
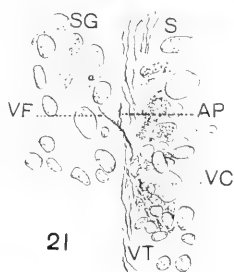
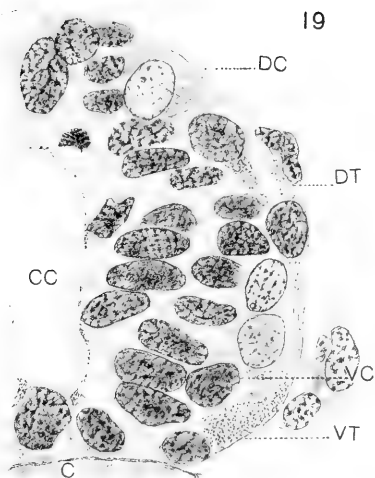
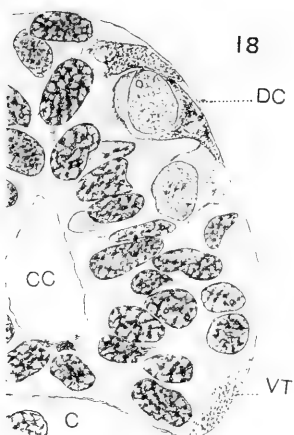
Figs. 22 to 23 From the same specimen (412, 5-2-3) as the last.

Fig. 24 From a specimen (408, 4-3-9) of the early swimming stage; method and sectioning the same as the last.

Figs. 25 to 26 From the same specimen (408, 4-3-10) as the last.

Fig. 27 From the same specimen (408, 4-3-11) as the last.

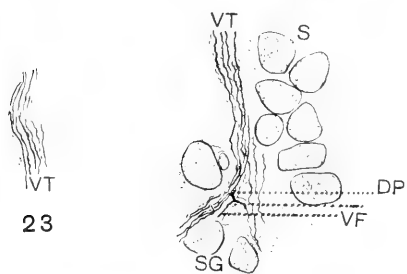
Fig. 28 From the same specimen (408, 3-2-7) as the last.



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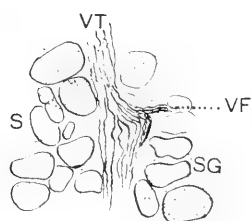
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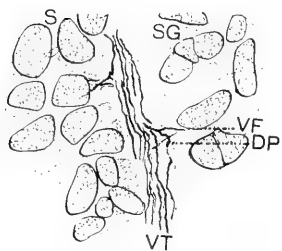
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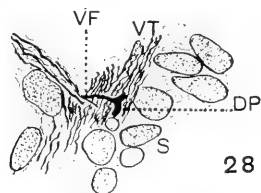
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THE NERVUS TERMINALIS IN THE ADULT DOG AND CAT

ROLLO E. McCOTTER

The Anatomical Laboratory, University of Michigan, Ann Arbor

FOUR FIGURES

In a previous communication the writer ('12) has shown the presence in mammals of two distinct groups of nerve fibers in the olfactory region which terminate in separate ganglionic masses on the surface of the olfactory bulb. These are the common olfactory fibers arising from the olfactory mucosa, and the vomeronasal nerves arising in the vomeronasal organ (Jacobson's organ). The former ramify in the glomeruli of the olfactory formation and the latter terminate in similar structures in the accessory olfactory bulb, or, as we might better designate it, the vomeronasal formation. In the same paper it was suggested that we might have to add to these a third group, the nervus terminalis which apparently differs essentially from the previous two groups. It was also mentioned that, judging from the dissections of adult dogs, it might be possible to demonstrate all three of these nerve groups in that animal. Since that time, as will be reported in the present paper, the writer has succeeded in clearly demonstrating in both the adult dog and cat the existence of a slender ganglionated nerve which in its position and character corresponds completely with the nervus terminalis as described by previous authors in lower forms. In other mammals it is either extremely small or is absent. The writer made a careful search for it in the opossum, rat, rabbit, guinea-pig and sheep and did not succeed in finding it.

Before describing the details of the connections of these nerves it may be well to remind the reader that the nervus terminalis was first described in 1894 by Pinkus who found it in Protop-

terus. Since that time it has attracted the attention of a large number of observers, among whom may be mentioned Locy ('05), Brookover ('08 '10), Sheldon ('09) and Brookover and Jackson ('11) with the result that the nerve has been described for nearly all groups of fishes. The presence of the *nervus terminalis* in Amphibia was studied by Herrick ('09) for the frog and by McKibben ('11) for Urodela. At the last meeting of the American Association of Anatomists at Cleveland, Johnston ('13) demonstrated reconstructions of pig embryos showing the presence there of a *nervus terminalis* which is connected peripherally with the vomeronasal nerves. He also reported having seen the same nerve in human and turtle embryos. For a complete history of the *nervus terminalis* the above references should be consulted.

My own observations are based in the first place upon six gross dissections of the adult dog and three of the cat; in the second place on the microscopical studies of these fibers after their removal; and thirdly on serial sections prepared to show the median wall of this part of the brain together with the coverings and the contained nerves.

In the preparation of the specimens for the purposes of dissection the same methods were used as previously described by the author ('12) for the identification of the vomeronasal apparatus. This method consists of dividing the head to one side of the mid-sagittal plane and immersing the larger part from twenty-four to forty-eight hours in Müller's fluid to which has been added 5 per cent glacial acetic acid. The nerves are then found to be toughened and differentiated in color, thereby facilitating their identification. The dissection was done under water with the aid of a binocular microscope. The first step in the procedure consists in the careful removal of the *falx cerebri* and the identification of the vomeronasal nerves which are then followed to the caudal border of the olfactory bulb where the *nervus terminalis* may be seen associated with it. Then the course of this nerve can be easily traced caudalward. The microscopical study of the dissected nerves was made by dividing the vomeronasal nerves at the dorsal border of the vomeronasal organ and at their entrance to the vomeronasal formation of the olfactory

bulb. The nervus terminalis was likewise divided where it entered the brain wall. These nerves were then removed from the specimen in one mass and stained in Van Gieson's hematoxylin-picro-fuchsin stain. Figures 3 and 4 represent drawings made from favorable portions of such a preparation. The serial sections used for this study consisted of a transverse series made from the medial half of the olfactory bulb and peduncle of the dog. The sections were stained in hematoxylin and congo red.

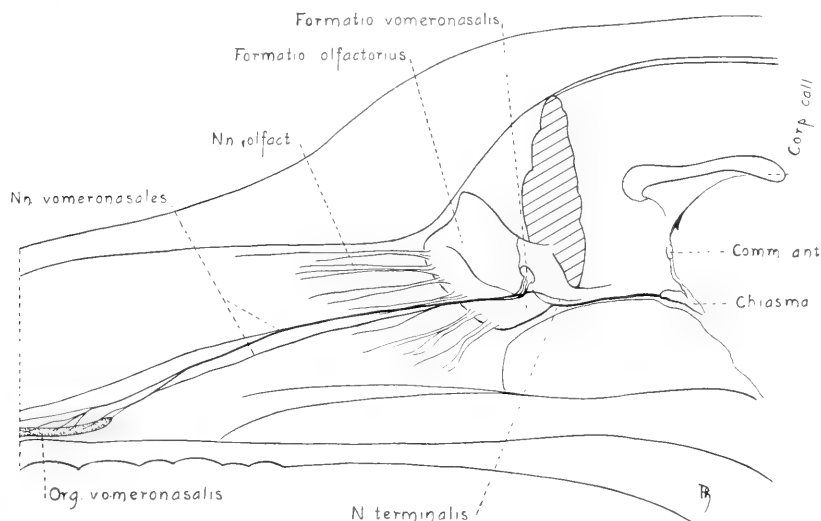


Fig. 1 A median section of the head of a dog with the frontal lobe of the brain, the nasal septum and the mandible removed, showing the course and termination of the nervus terminalis and its connection with the vomeronasal nerves. Natural size.

The relation of these structures as seen in the dissections of the dog are shown in figure 1. The vomeronasal nerves upon passing through the cribriform plate course almost horizontally across the narrow medial aspect of the olfactory bulb to its caudal border where they break up in a fine plexus and turn lateralward upon the dorsal aspect of the olfactory bulb. Connected with this plexus are several small bundles that usually unite into a single trunk which extends caudoventralward on the

medial surface of the olfactory peduncle where it appears to enter the brain substance some distance from the olfactory bulb. In one specimen, however, instead of entering the brain wall on the medial surface of the olfactory peduncle the nerve passed across this surface and apparently entered on the ventral surface of the peduncle. A few small filaments which seem to be connected with the olfactory nerves join the nervus terminalis just after its separation from the vomeronasal nerves.

Upon microscopical examination of the specimen of the vomeronasal nerves and the nervus terminalis, stained in mass, there were observed large oval, fusiform and cone-shaped nerve cells with granular cytoplasm and large spherical nuclei with prominent nucleoli. These cells were present in considerable numbers and were found either grouped around the nerve or embedded between its fibers causing a fusiform enlargement of the nervus terminalis immediately after its fibers have separated from the vomeronasal nerves.

The examination of the transverse sections of the olfactory bulb of the dog confirmed the above mentioned observations. The vomeronasal nerves were easily recognized and followed to the vomeronasal formation. The filaments of the nervus terminalis can be seen separating from the vomeronasal nerves. A group of ganglion cells enclosed in a capsule and attached to the combined filaments was followed through eight consecutive sections. In this particular series, the filaments having been slightly torn in the preparation of the block, the nerve could not be traced into the brain substance.

The vomeronasal nerves of the cat (fig. 2) course upward and backward in three separate bundles on the medial surface of the olfactory bulb to the caudal border of this surface where they unite into a loose plexus and turn outward to end in the vomeronasal formation. Several small filaments separate from the above mentioned plexus and unite into a single strand which courses caudalward on the medial surface of the olfactory peduncle where it apparently enters the brain substance in the region of the arcuate fissure. The nervus terminalis gradually decreases in size from its connection with the vomeronasal nerves to its termination

in the brain cortex. This is due to three or four small filaments that leave the nerve at intervals and apparently enter the brain substance at different points along the course of the nerve.

Upon microscopical examination of the vomeronasal nerves and the nervus terminalis, which were dissected off and stained in mass, there can be seen a small spindle-shaped ganglion composed of about 200 cells causing an enlargement of the nerve shortly after its fibers have separated from the vomeronasal nerves.

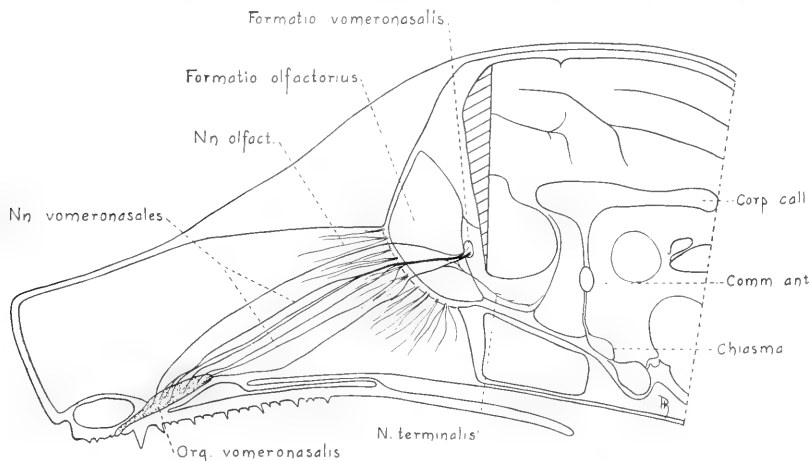


Fig. 2 Represents the median section of the head of a cat with the frontal lobe of the brain, the nasal septum and mandible removed, showing the course and termination of the nervus terminalis and its connection with the vomeronasal nerves. $\times 1\frac{1}{2}$.

Ganglion cells are scattered around the nerve and between its fibers throughout the greater part of its course. Figure 3 represents a camera lucida drawing of a part of the nervus terminalis proximal to the ganglion showing the distribution of these cells. On a careful examination of the septal portion of the vomeronasal nerves within the nasal cavity a clump of nerve cells was found on each of two of its seven filaments. They lie at the side of the nerve and attached to it just dorsal to the vomeronasal organ. These ganglion cells cannot be the cell bodies of the vomeronasal nerve filaments because it has long been known

that the vomeronasal nerves are the axis cylinder processes of the sense cells of the mucosa of the vomeronasal organ and arise in the same manner as the olfactory nerve filaments from the sense cells of the olfactory mucosa. It is quite evident, therefore, that these clumps of ganglion cells belong to the nervus

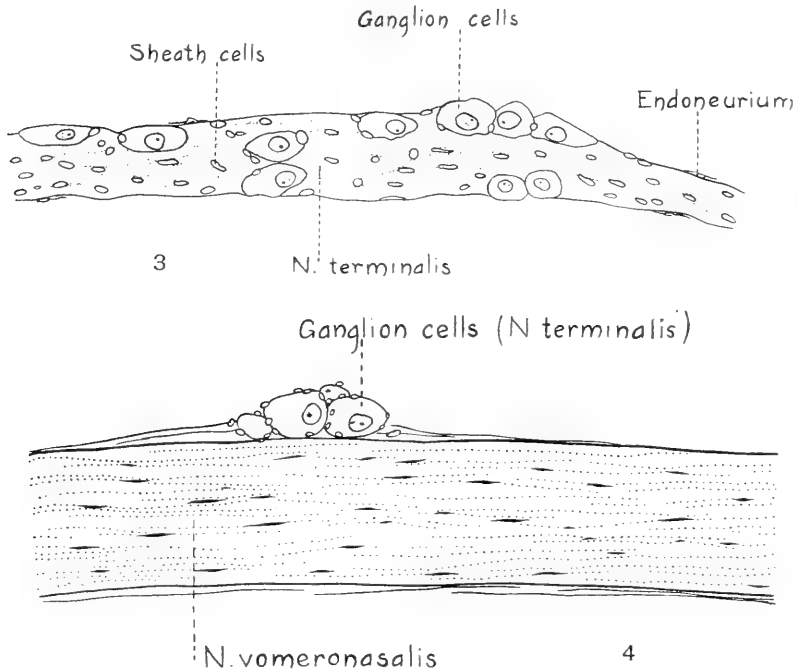


Fig. 3 A camera lucida drawing of a favorable portion of the nervus terminalis of the cat proximal to its ganglion to show the characteristic distribution of the nerve cells. $\times 200$.

Fig. 4 A camera lucida drawing of one of the filaments of the vomeronasal nerves of the cat, taken not far from its origin from the vomeronasal organ. The clump of ganglion cells attached to it indicates that fibers of the nervus terminalis extend into this region. $\times 200$.

terminalis, the filaments of which extend into the nasal cavity along with several filaments of the vomeronasal nerves and apparently terminate within or very close to the vomeronasal organ.

From the foregoing description it is evident that there is normally present in the adult dog and cat a ganglionated nerve

connected with the vomeronasal nerves on the one hand and apparently with the forebrain on the other, having thereby the same morphological relations in these mammals as is described for the nervus terminalis of lower forms. Therefore we believe that we are justified in considering this nerve the nervus terminalis.

In conclusion it may not be out of place to add a word regarding the terminology of this region. One source of confusion in terminology is the use of terms applicable to surface appearances but not applicable to the same structures as they appear in sections made through them. It is still worse when the term used applies to the appearances in some animals but not in others. Manifestly the ideal terminology should express, as far as possible, the function and connections of the parts concerned. Unfortunately, however, in some cases we have to apply a terminology before the function and even the connections are known. In the region we have been considering the terms olfactory bulb and olfactory stalk are widely accepted and have been found very convenient in the description of the surface anatomy of these parts. In a previous paper it was suggested in a tentative way that we call the accessory olfactory bulb the 'vomeronasal tubercle.' It is true that it forms an elevation on the olfactory bulb of mammals, but in many of the lower forms (reptiles), however, it is described as a pit or fossa. It therefore seems feasible to substitute the term 'formatio vomeronasalis.' This term is consistent with 'formatio olfactoria,' the receptive ganglion of the ordinary olfactory nerves, which is in common use. Then if we apply the term 'olfactory bulb' in its true sense to include the entire bulbous enlargement of the olfactory evagination, it will be found composed of four separate and distinct parts: (1) the formatio olfactoria where the ordinary olfactory fibers terminate; (2) the formatio vomeronasalis which is the receptive center for the vomeronasal nerves; (3) the pars bulbaris of the lateral olfactory cortex which is to a greater or less extent covered in and attenuated by divisions 1 and 2, and which includes also, the over-lying layer of olfactory tract fibers as well as the deeply placed layer of the pars olfactoria of the anterior commissure;

(4) the ventricle of the bulb with its lining ependyma. Based upon the classification given by Kölliker, each of the subdivisions of the olfactory bulb is then composed of the following laminae:

Bulbus olfactorius	{	(1) Formatio olfactoria
		(A) Fila olfactoria
		(B) Stratum glomerulorum
		(C) Stratum griseum
		(a) Stratum moleculare
		(b) Stratum mitrale
		(D) Stratum granulare
		(2) Formatio vomeronasalis
		(A) Fila vomeronasales
		(B) Stratum glomerulorum
		(C) Stratum griseum
		(a) Stratum moleculare (including mitral cells)
		(3) Cortex olfactorius lateralis, pars bulbaris
		(A) Stratum plexiforme (including tract. olf. lat.)
		(B) Stratum pyramidale
		(C) Stratum fibrosum (including comm. ant., pars olfact.)
		(4) Ependyma and Ventriculus bulbi

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THE EYE-MUSCLE NERVES IN NECTURUS

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EIGHT FIGURES

Information concerning the eye-muscle nerves in Amphibia seems abundant. For Salientia these nerves have been partially described by Arnold ('94) and Strong ('95) and the relations in the adult frog given by Gaupp ('99). In Urodela descriptions are fragmentary for many species, but in Amblystoma, Herrick ('94); Spelerpes, Bowers ('00); Amblystoma, Coghill ('02); Triton, Coghill ('06) and Amphiuma, Norris ('08); the origin, course, relations and distribution of these nerves have been quite fully set forth. In Proteida, the classical description of Fischer ('64) in Menobranchus (Necturus) is incomplete, as is that of Osborn ('88), for Proteus, the extremely small size of the trochlear and abducent nerves making them difficult even to identify. In his study of the brain of Necturus, Kingsbury ('95) gives descriptions of the origins of all the eye-muscle nerves. The present contribution may serve to supplement the observations of Kingsbury by adding descriptions of all the eye-muscle nerves in Necturus from their origins to their distributions in the orbit.

MATERIAL AND METHOD

The specimens of *Necturus maculosus* Rafinesque used were adult animals, varying in length from 36 cm. to 42 cm., supplied by Alex Nielsen, Venice, Ohio.

The extremely small size of the eye-muscle nerves makes them difficult to study in fresh or preserved material; but by staining with methylene blue, intra-vitam, and careful dissection under a stereobinocular microscope, the origin, course and distribution of each of these nerves may be quite easily demonstrated.

The method used is with some modification that given by Wilson ('10). The animals are tied by the legs to a board, the root of the tail being held immovable by a wooden clamp. The tail is cut off and after bleeding the stain is injected through the caudal artery. A 0.066 to 0.075 per cent solution of methylene blue (Grübler's "med. pur." or "rect., nach Ehrlich") in salt solution was found most useful.

Methylene blue (0.5 per cent in water)	13-15 cc.
Salt solution (0.75 per cent in water)	87-85 cc.

About 200 cc. of the stain is usually injected into an animal of 40 cm., outlet being furnished by the caudal vein and by cutting the tips of the gills to which clamps are applied when the vessels are well filled. Pressure is obtained by gravity and for a good injection of vessels of the head a pressure is necessary which is sometimes sufficient to rupture abdominal capillaries. After the vessels are well filled and the outlets cut off, the animal remains untouched for three to five minutes. The head is then cut off, the lower jaw removed and the region to be studied moistened with salt solution and exposed to the air. After a successful injection color appears in the eye-muscle nerves almost immediately on exposure to the air. The dye in the other nerves is oxidised somewhat later.

For fixation, if permanent preparations are desired, a cold solution of ammonium molybdate (8 per cent in water) is used. This is applied in an ice box for eighteen to forty-eight hours. (Some material, thus fixed, has been kept successfully, for purposes of dissection, in 4 per cent neutral formaldehyde—9 parts water, 1 part, 40 per cent formaldehyde—for four or five days without total loss of the stain in the nerves). The molybdate is now removed by washing in cold running tap water or by numerous changes of cold water in the ice box and the tissue then transferred to several changes of 96 per cent alcohol in the ice box and then to absolute alcohol. Clearing is accomplished in xylol and the tissue mounted in balsam or embedded in paraffin. If kept in the dark, preparations will keep for several years. For further details concerning this method, see Wilson ('10).

About twenty-five animals have been used in this study and the eye-muscle nerves on both sides of twelve of them thoroughly dissected. The rapidity with which the dye in the nerves is oxidised makes impossible the completion of a dissection of the fresh material which involves several hours, so that the dissections were continued after fixation, in a cold solution of ammonium molybdate and the relations of some of the larger structures established after the material was transferred to neutral formaldehyde.

The drawings shown in figures 1 and 2 were made from brains of *Necturus* fixed in formaline-Zenker's fluid, in which neutral formaldehyde (40 per cent) is substituted for the acetic acid ordinarily used with Zenker's fluid. Measurements indicate that this fluid produces in this brain less change in the size, shape and relations of parts than other solutions most often used for the fixation of whole brains. In the brain of *Necturus* there occurs usually a very slight swelling in the fore-brain (2.5 to 3 per cent); in the mid-brain and medulla oblongata no marked changes in size due to fixation have been noted after use of this fluid. In the fixation of the brain of *Necturus* it is essential that the brain be left in the skull and removed after hardening. This is particularly well illustrated by the appearance of the hypothalamus, hypophysis and saccus vasculosus after various treatments (fig. 2). Failure by several authors to recognise the saccus vasculosus in tailed *Amphibia* has been due evidently to improper conditions during fixation. If the meninges and blood vessels at the caudal end of the hypophysis are cut, the attachments of the hypophysis and the saccus vasculosus shrink and shrivel during fixation so that the hypophysis comes to lie ventral to the hypothalamus and the lateral dilations of the saccus vasculosus show no longer the relations observed in the fresh condition. In order to insure for the fixing fluid ready access to the brain, the part of the parasphenoid bone which forms the floor of the cranium should be removed from the cephalic tip of the hypophysis to the middle of the olfactory tracts. The bone should be left intact over and caudal to the hypophysis. Removal of the roof of the cranium may disturb the attachment

of the paraphysis, causing more marked change in the form of the fore-brain than occurs when a part of the parasphenoid is removed. Fixation of the brain of any amphibian may preserve more closely the normal relations as seen in the fresh condition if the precautions above mentioned are heeded.

Variability

The optic apparatus in *Necturus*, when compared with that in some other tailed Amphibia, seems poorly developed. The habits and reactions of *Necturus*, so far as known, also indicate the comparatively slight functional importance of the eye. Considerable variability is noted in the eye-muscle nerves in their passage from the brain to the orbit and in their ultimate branches. The relations of the trunks of the oculomotor and abducent nerves in *Necturus* seem fairly constant, but the trochlear nerve shows some remarkable differences in different individuals and often varies greatly on the two sides of the same head. Such variability in the course and relations of the eye-muscle nerves in Amphibia has previously been noted by Coghill ('02 and '06) and by Norris ('08). As pointed out by these authors, conclusions concerning cranial nerves in Amphibia can not be drawn accurately from a study of single or even several specimens.

In no two of the twenty-four eyes of the twelve individuals studied carefully do the eye-muscle nerves appear exactly alike. The descriptions and figures which follow illustrate the average condition, special note being made of some of the most interesting variations.

N. oculomotorius

The oculomotor nerve is formed by two or three bundles of fibers which spring from the ventral surface of the mid-brain about 0.6 mm. cephalad to the lateral pouches of the medulla oblongata and about 0.4 mm. from the midline. The nerve runs directly laterad above the saccus vasculosus (fig. 2) and inclining slightly cephalad reaches its foramen which lies about 3.7 mm. caudad to the optic foramen in the parietal bone (fig. 4). The foramen for the oculomotor nerve lies usually about 1.1 mm.

dorsal to the optic foramen. Running now for a distance of about 3 mm. in its bony canal, which inclines sharply cephalad, the oculomotor nerve emerges from the parietal bone about 4.8 mm. caudad to the emergence of the optic nerve; a point about at the level of the preoptic nucleus in the brain. Here the nerve lies in the trabecular cartilage immediately ventral to the r. ophthalmicus profundus V, and passing cephalad and very slightly laterad in the same relation, divides into its ventral and dorsal rami about 0.7 mm. cephalad to the foramen of exit of the optic nerve from the parietal bone; a point about at the level of the entrance of the olfactory tract in the olfactory bulb.

The dorsal ramus now passes dorsad to the optic nerve and running medial to or piercing the r. ophthalmicus profundus V, reaches the common origin of the recti muscles (figs. 5 and 7). Here it applies itself closely to the superior rectus muscle and breaks up on the dorso-medial surface of this muscle about its middle (fig. 5).

There are usually present one or two small twigs, containing two or three fibers, which leave the dorsal ramus of the oculomotor nerve to enter the bulbar fascia (fig. 5). Occasionally some of these fibers may be traced to the eyeball but most of them are lost among the blood vessels of the bulbar fascia.

The ventral ramus of the oculomotor nerve, running immediately ventral to the r. ophthalmicus profundus V and ventrolateral to the optic nerve, reaches the common origin of the recti muscles and passes directly out on the ventral face of the inferior rectus muscle (fig. 6). Quite frequently the ventral ramus enters the inferior rectus muscle and in several cases was seen to lie entirely dorsal to this muscle (fig. 7).

During its passage cephalo-laterad on the inferior rectus muscle, several twigs rise from the ventral ramus of the oculomotor nerve which enter this muscle and, having reached its ventromedial edge, the ventral ramus splits into two branches one of which runs directly to the medial rectus muscle, and the other, passing along the medial border of the inferior rectus finally enters the inferior oblique muscle near to its insertion on the eyeball (fig. 6).

When the ventral ramus of the oculomotor nerve reaches the common origin of the recti muscles there usually arise from it one or two small twigs of two to four fibers, which, passing into the bulbar fascia, finally reach the eyeball. Very often one of these branches applies itself to the sheath of the optic nerve and thus gains the eyeball (fig. 7). In several cases these twigs have been seen to enter either the inferior oblique or the medial rectus muscle; but usually one at least is present which reaches the eyeball and this very often in company with the sheath of the optic nerve.

In three of the eyes examined there was present a small twig from the ventral ramus of the oculomotor nerve which entered the lateral rectus muscle along with the abducent nerve.

N. trochlearis

The trochlear nerve rises from the caudal border of the dorsal surface of the mid-brain and passes ventro-laterad and slightly cephalad, lying in the angle between the mid-brain and the rostral border of the medulla oblongata and cerebellar commissure (figs. 1, 3 and 8). The root of the nerve rises after decussation immediately cephalad to the cerebellar commissure and contains usually sixteen to twenty-four fibers. A few fibers have been observed which seem to enter the nerve uncrossed. These rise from the caudal border of the optic tectum about 0.1 mm. from the midline and at once seem to enter the main trunk of the nerve. Four to eight such fibers usually appear on either side in preparations stained with methylene blue. On careful examination under high magnification, these fibers seem to be larger fibers than those which make up the trochlear nerve and to belong to the mesencephalic root of the trigeminal nerve which lies here beneath the trochlear nerve. Two or three smaller fibers from the tectum have been observed which appear to enter the trochlear nerve uncrossed.

The work of Tczer and Sherrington ('10) shows that in mammals the eye-muscle nerves contain sensory fibers (proprioceptive). If a similar arrangement exists in *Necturus* and the mesencephalic root of the trigeminal nerve is a sensory root, we may

have here, just after its decussation, the reception by the trochlear nerve of these sensory fibers; but further investigation is required to establish these relations in *Necturus* with certainty.

Figure 8 (right side) shows a case in which the trunk of the trochlear nerve runs at first directly caudad and then laterad, splitting into two bundles, one of which runs through the choroid plexus of the fourth ventricle. One or two fibers are sometimes given off the main trunk of the nerve in its passage laterad, to the lateral processes of the choroid plexus.

Passing directly caudad over the cerebellar commissure and into the choroid plexus of the fourth ventricle there are quite constantly present three to six large medullated fibers which seem to leave the main trunk of the trochlear nerve. These fibers, previously noted by Kingsbury ('95), are lost among the blood vessels of the choroid plexus. The connections of these fibers have not been demonstrated.

There have been observed in the dura mater in many specimens fibers similar in size to those just described. Such fibers have been traced from the choroid plexus of the fourth ventricle cephalad to the pineal region. Their origin has not been determined.

Having reached the dura mater, at this point quite closely adherent to the skull, the trochlear nerve turns, and passing usually about 1 mm. dorsal to the oculomotor nerve runs cephalad in the dura of the lateral wall of the cranial cavity (fig. 3). Passing now dorsal to the optic nerve, the trochlear nerve runs in the dura to a point usually about 0.5 mm. cephalad of the olfactory bulb and, forming a loop, returns to its foramen which in the majority of cases lies in the parietal bone about 2.5 mm. dorsal to the optic foramen. Quite frequently the foramen for the trochlear nerve may lie 0.2 mm. cephalad to that for the optic nerve or it may lie as much as 0.3 mm. behind it. In several cases the two foramina were in the same transverse plane but they are always separated by almost the entire depth of the cranial cavity.

One animal was examined in which, on one side, the foramen for the trochlear nerve in the parietal bone, was 0.6 mm. caudal to the lateral angle of the olfactory foramen.

In two cases, the trochlear nerve running cephalad in the dura mater, left the cranial cavity through the olfactory foramen, turning then sharply laterad to reach the superior oblique muscle.

During its course inside the cranial cavity the trunk of the nerve frequently splits into several fascicles. This splitting usually occurs in the region of the loop of the nerve or soon after its origin from the brain and before the lateral cranial wall is reached.

After reaching its foramen the trochlear nerve runs laterad and cephalad in its canal in the parietal bone for a distance of about 2 mm. (fig. 3). Reaching thus the dorsal face of the lateral process of the parietal bone, the nerve runs cephalad close to the bone from which the temporal muscle is arising. Having arrived at the cephalo-medial angle of the orbit, the nerve swings laterad and breaks up on the dorsal face of the superior oblique muscle.

In one animal the nerve on the right side joined the oculomotor nerve, leaving the cranium through the oculomotor foramen. The fibers of the two nerves intermingled so that it was impossible to separate them. In this case the superior oblique muscle was innervated by a branch of the dorsal ramus of the oculomotor nerve, presumably by trochlear fibers.

An anastomosis of the trochlear nerve has been observed in several cases with the medial branch of the r. ophthalmicus profundus V inside the orbit (fig. 5); and in several cases a twig from the same branch of the trigeminal nerve has been seen to enter the sheath of the superior oblique muscle near to its insertion on the eyeball.

N. abducens

The abducent nerve is formed by two or three twigs which rise from the ventral face of the medulla oblongata about 0.3 mm. from the midline and 0.5 to 0.8 mm. cephalad to the root of the glossopharyngeal nerve (fig. 2). The abducent nerve is the smallest of the cranial nerves and contains usually ten to fifteen fibers. Running at first directly laterad, the nerve swings cephalad to the ventral surface of the root of the facial and acoustic

nerves and passing forward in the sheath of these nerves comes to lie on the ventral face of the Gasserian ganglion of the trigeminal nerve. Here the abducent nerve lies in the connective tissue sheath of the Gasserian ganglion, no interchange of fibers ever having been observed between the abducent nerve and any part of the trigeminal. Passing now slightly ventrad, the abducent nerve soon enters the temporal muscle which at this point takes its origin from the lateral surface of the trabecular cartilage. Running in this muscle just ventro-laterad to the r. ophthalmicus profundus V, the abducent nerve reaches the common origin of the recti muscles and then swings laterad to enter the ventro-caudal face of the lateral rectus muscle (figs. 4, 5, 6 and 7).

In four of the eyes dissected, a small twig has been observed running to the sheath of the optic nerve from the abducent nerve as it breaks up in the lateral rectus muscle (fig. 5). In one case this was a large twig of five or six fibers, in the others only two or three fibers were concerned.

Nn. ciliares

There is constantly present a branch of the r. ophthalmicus profundus V, which enters the sheath of the optic nerve (figs. 5 and 7). This fascicle, containing usually about ten fibers, leaves the lateral border of the r. ophthalmicus profundus V just before the orbit is reached, and running dorsal to the lateral rectus muscle, applies itself to the dorso-caudal face of the sheath of the optic nerve. Many of the fibers of this twig reach the eyeball.

Mention has already been made, in the descriptions of the oculomotor and abducent nerves, of twigs from these nerves entering the bulbar fascia and the sheath of the optic nerve. Some of these twigs reach the eyeball.

In none of the specimens examined has there been found any group of cells corresponding to the ciliary ganglion. The condition in *Necturus* may be similar to that in *Squalus acanthias* as reported by Dr. H. D. Senior at the meeting of the American Association of Anatomists, December, 1912. Here no ciliary ganglion is said to exist, the motor ciliary nerves containing,

probably, only vaso-motor fibers. In *Squalus acanthias*, according to Senior, physiological examination reveals great simplicity of accommodation; a fact correlated with the absence of the ciliary ganglion. No similar experiments with *Necturus* are known to the writer.

SUMMARY

In *Necturus* all the eye-muscle nerves have been demonstrated from their origin to their distribution in the orbit.

The trochlear nerve shows frequent variations in its intracranial course and in the position of its foramen in the parietal bone.

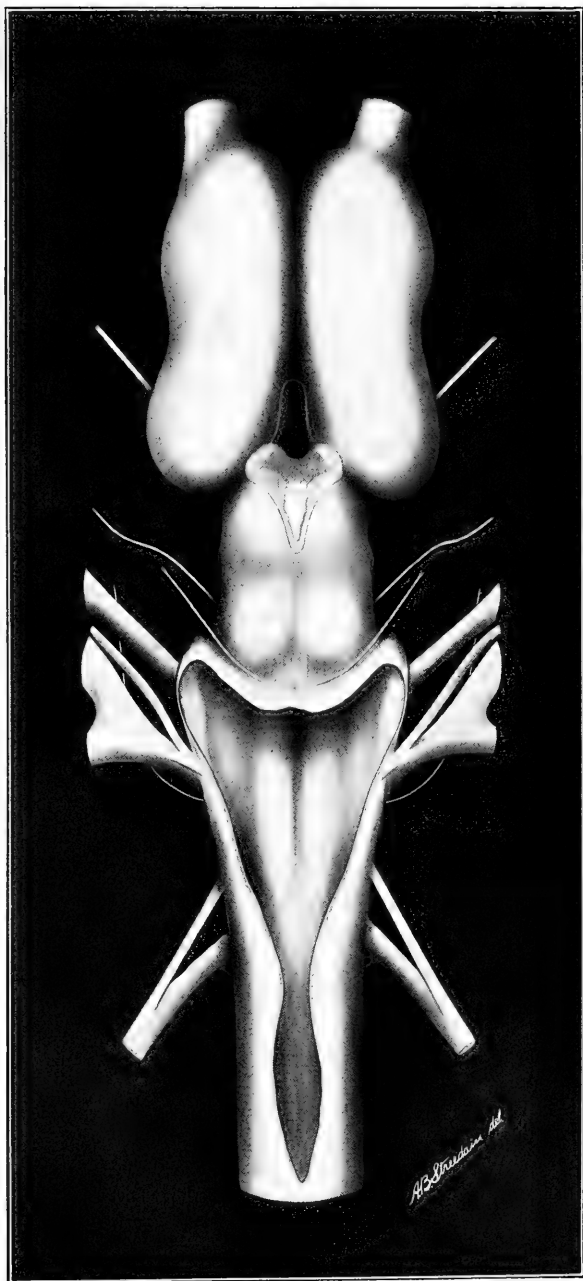
No group of cells corresponding to the ciliary ganglion has been found, but there are quite constantly present twigs from the r. ophthalmicus profundus V, the oculomotor and the abducent nerves which enter the bulbar fascia and the eyeball. These twigs most often apply themselves to the sheath of the optic nerve.

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Fig. 1 Drawing of the dorsal aspect of the brain of *Necturus* fixed in formaline-Zenker's fluid and removed from the cranium after fixation. The choroid plexus of the fourth ventricle and the paraphysis have been removed. $\times 6$.



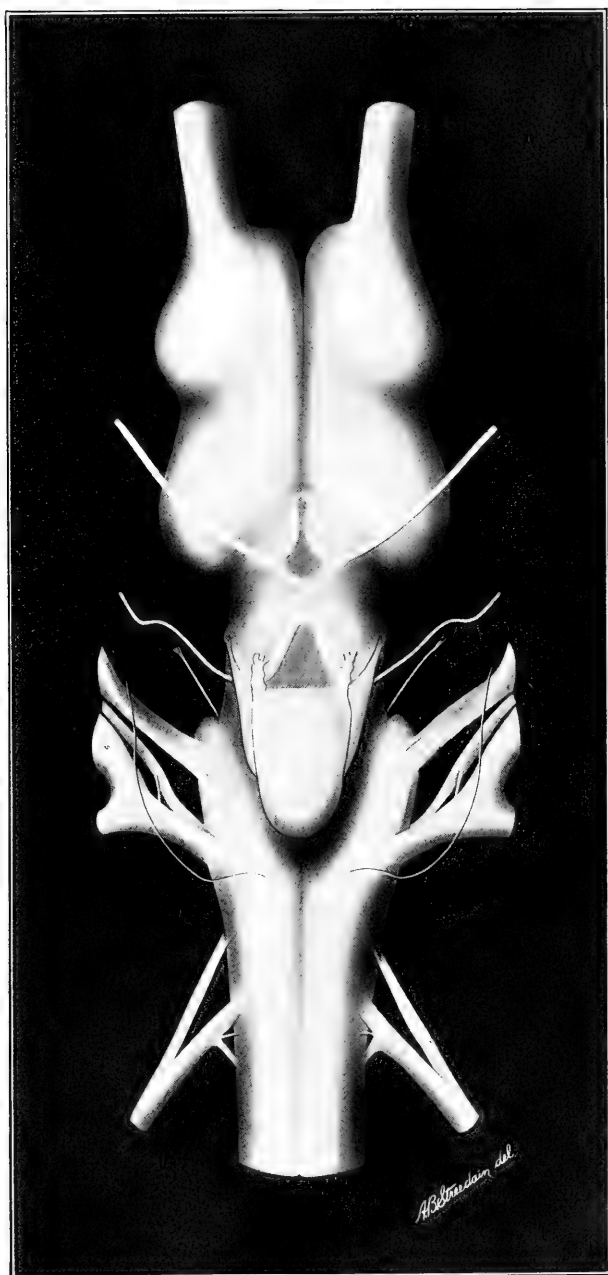


Fig. 2 Drawing of the ventral aspect of the brain of *Necturus*. $\times 6$

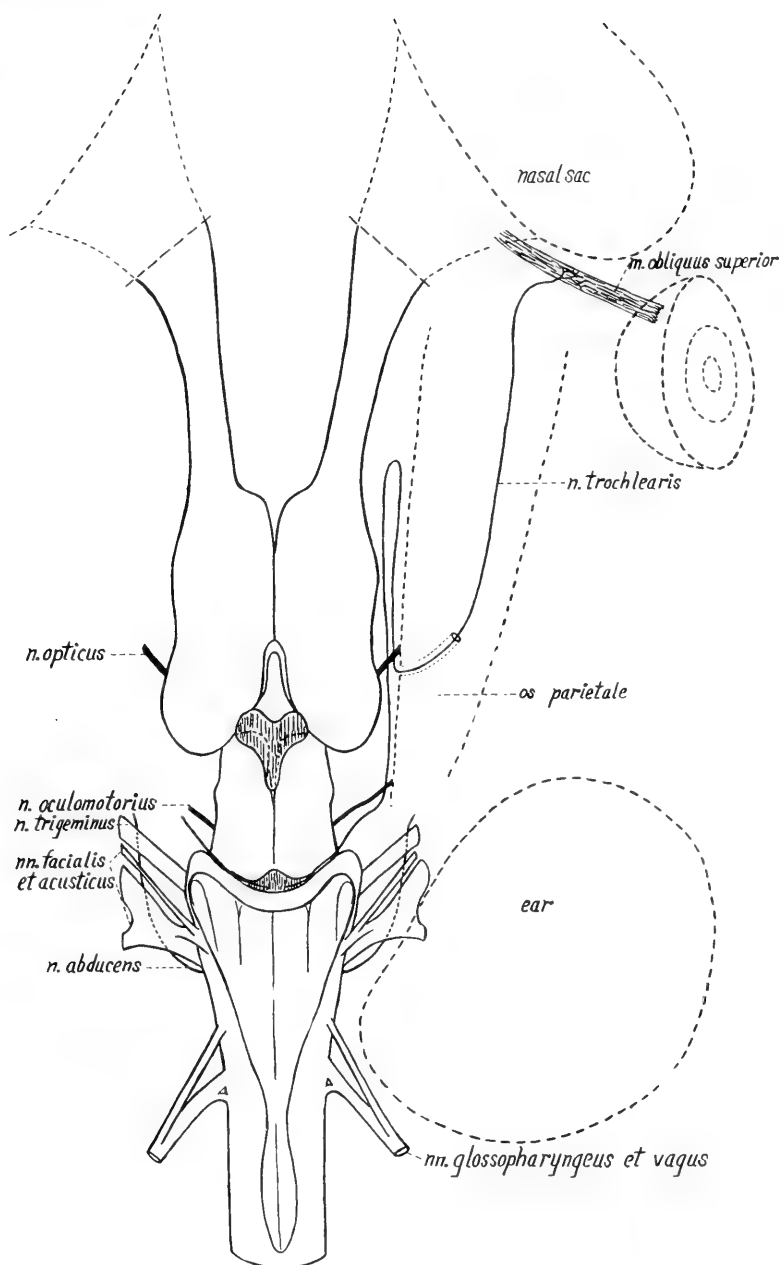


Fig. 3 Dorsal aspect of the brain of *Necturus* showing relations of the nasal sac, eye and ear and the course of the trochlear nerve. $\times 4.5$

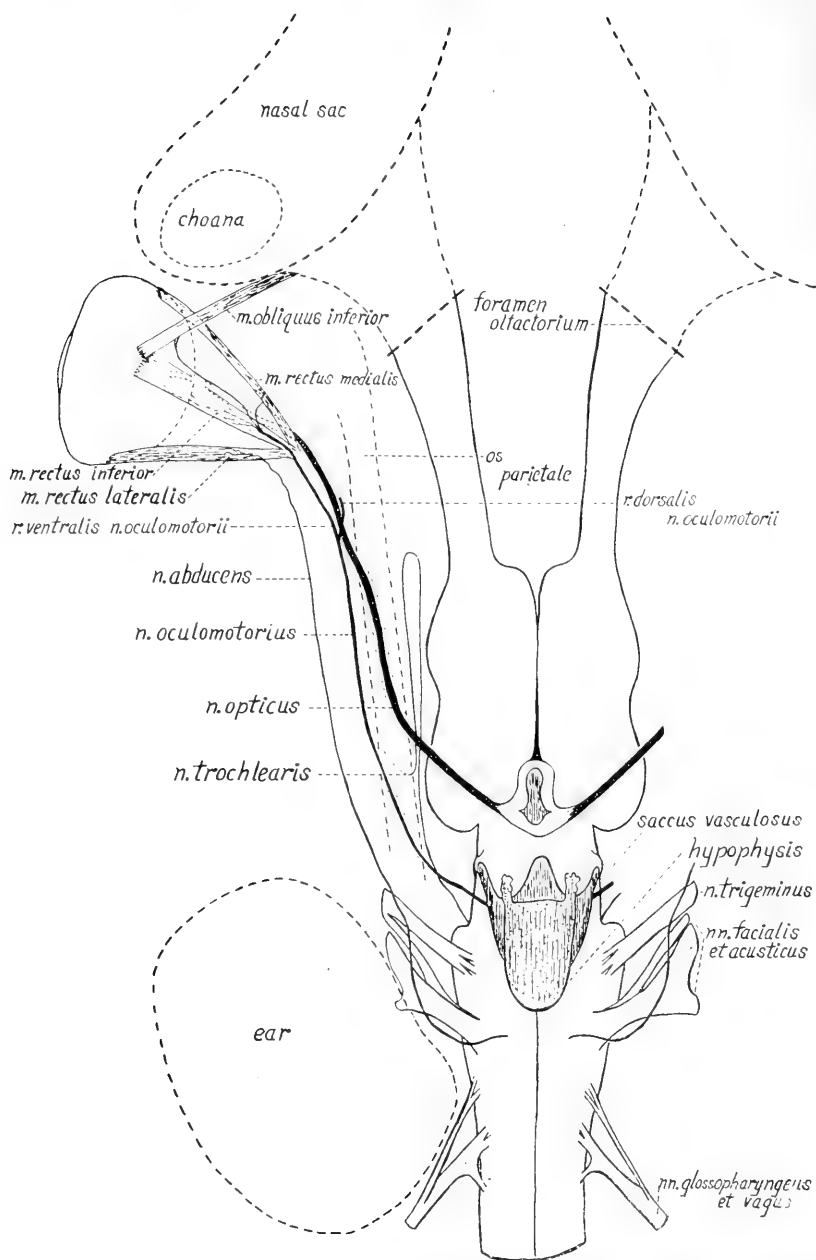


Fig. 4 Ventral aspect of the brain of *Necturus* showing relations of the nasal sac, eye and ear and the course of the optic, oculomotor and abducent nerves.
 × 4.5.

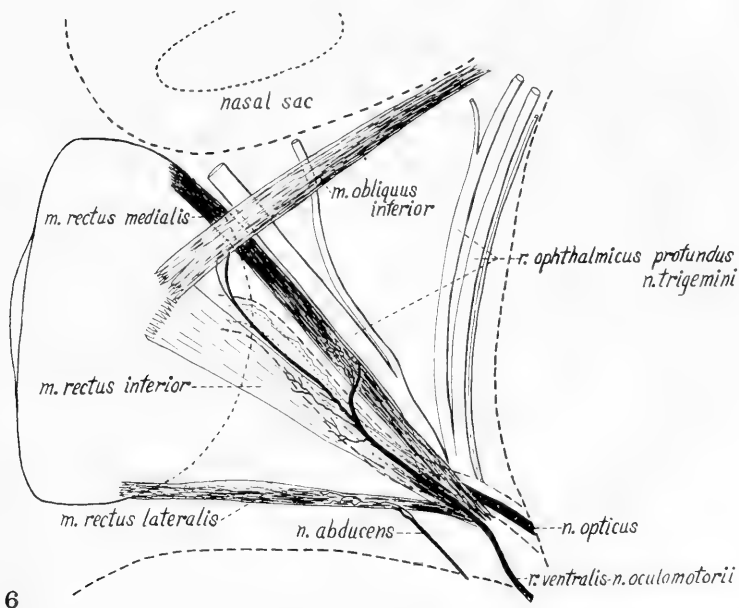
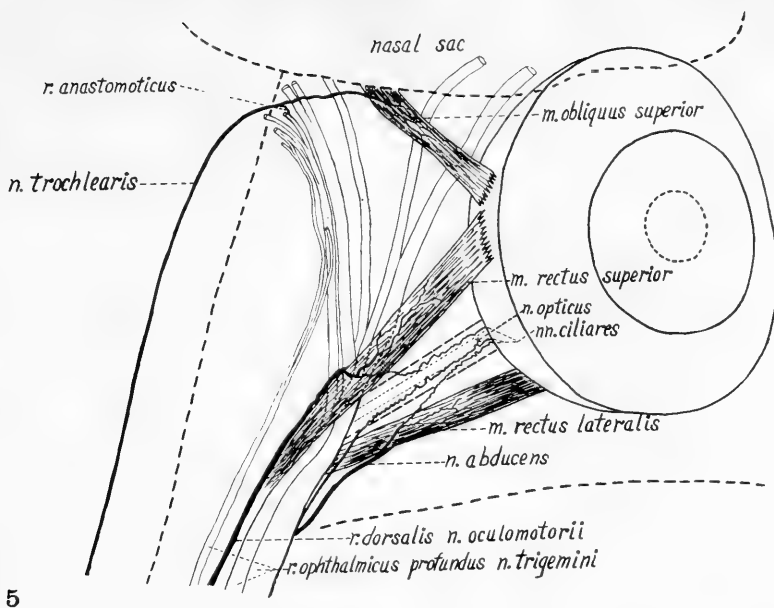


Fig. 5 Dorsal view of the right orbit showing termination of the trochlear and abducent nerves and of the dorsal ramus of the oculomotor nerve. $\times 10$.

Fig. 6 Ventral view of the right orbit showing termination of the ventral ramus of the oculomotor nerve. $\times 10$.

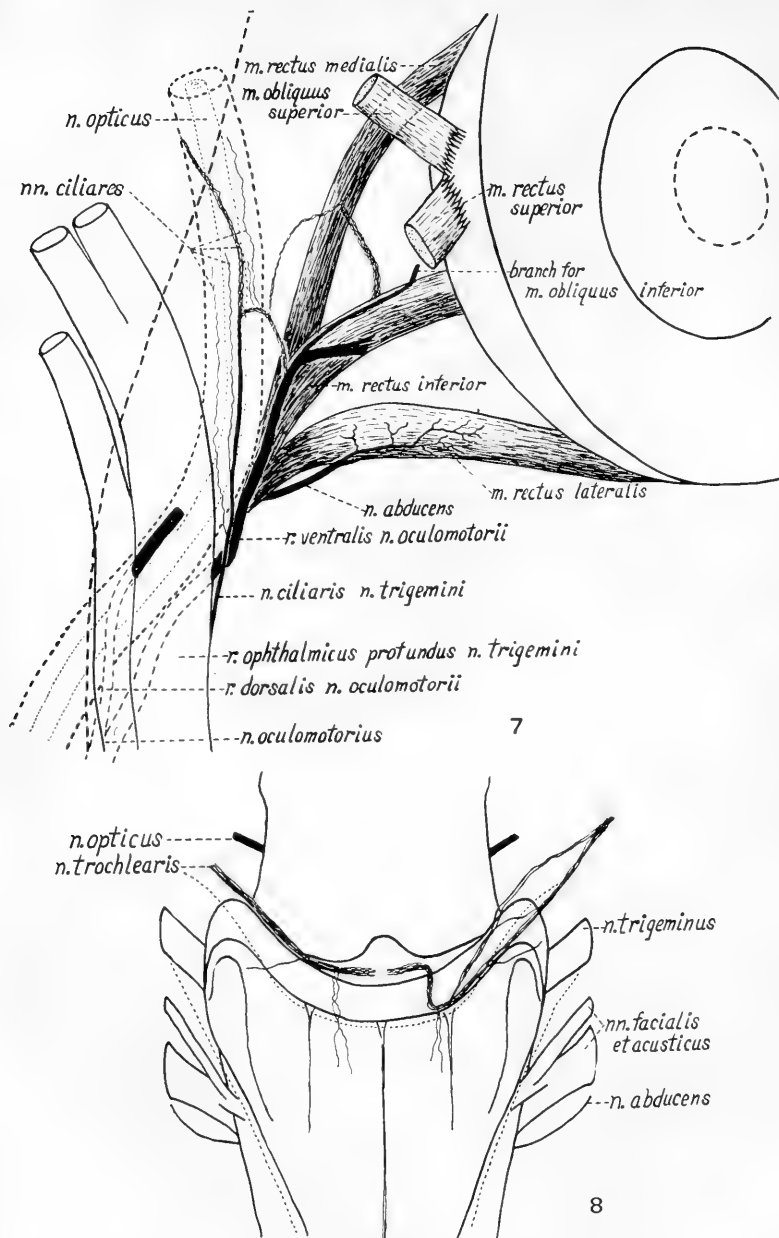


Fig. 7 Dorsal view of the right orbit. The superior rectus and the superior oblique muscles have been cut. The optic nerve has been cut close to the eyeball and turned medially so as to show the ciliary nerves on its caudal surface. This drawing shows also a case in which the ventral ramus of the oculomotor nerve is dorsal to the inferior rectus muscle (compare fig. 6). $\times 15$.

Fig. 8 Dorsal view of the mid-brain and medulla oblongata showing relations of the trochlear nerve. On the left side the most usual relations are shown. On the right side appear the relations in a case in which the nerve was split into two fascicles, the main trunk of the nerve passing caudad over the cerebellar commissure to the choroid plexus of the fourth ventricle. The dotted lines indicate the edges of the choroid plexus. The connections of the fibers which appear coming from the trochlear nerve to the choroid plexus have not been determined. $\times 9$.

ON THE INNERVATION OF THE DIGESTIVE TUBE

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FIVE FIGURES

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INTRODUCTION

A review of the literature bearing on the morphology of the sympathetic nervous system shows that the histological characters of the sympathetic neurones are quite definitely known. Ever since the introduction of the Golgi method, sympathetic neurones have been described. More recently our knowledge of the sympathetic neurones has been furthered by the use of the methylen blue method of Dogiel, the silver reduction method of Cajal and the modified silver reduction method of Bielschowsky.

Recent investigators, notably Cajal, Dogiel, Michailow and Müller, have described the neurones in the various parts of the sympathetic nervous system in the Mammalia, including man, in great detail. Each of these investigators has attempted a more or less complete classification of sympathetic neurones according to morphological type. Thus Cajal ('05, '06), whose

classification is accepted by Müller ('12), recognizes three distinct types. Dogiel and Michailow both recognize a greater diversity of sympathetic neurones, the latter ('11) claiming to be able to recognize as many as nine distinct types.

In general it may be said that the neurones in the various parts of the sympathetic nervous system show certain distinctive histological characters. Müller ('12) expressed the opinion, however, that all sympathetic neurones are fundamentally of the same morphological type, but differ somewhat in the structure and disposition of their dendrites according to the demands of the functions of the organs innervated by them. To quote: "Zusammenfassend glaube ich annehmen zu dürfen dass schließlich der Grundtypus aller Zellen des vegetativen Nervensystems derselbe ist, dass sich aber die Zellen beziehungsweise ihre Dendriten unter den verschiedenen Ansprüchen welche die Function des betreffenden Organs an sie stellt, verschieden gestalten." This opinion is of interest in view of the fact that, as pointed out by the writer in an earlier paper ('10), all sympathetic neurones arise from cells which are the descendants of the 'germinal' cells (Keimzellen) of His, that is, they all have a common origin.

The myenteric and the submucous plexuses with their component elements were described by Cajal as early as 1893. By the use of his own modification of the Golgi method, he was able to determine that all the neurones in these plexuses are multipolar and that the fibers are of the non-medullated variety. He believed, however, that all the protoplasmic processes of these neurones are essentially axones.

Cajal's observations were substantially corroborated by the work of Kölliker ('94) who suggested, furthermore, that these multipolar neurones might provide the apparatus for local reflexes. He says: "So könnten beispielsweise Zellen des Meissner'schen Geflechts mit oberflächlichen Ausläufern in den Darmzotten gewisse Erregungen aufnehmen und mit andern Ausläufern auf die Muskelfasern der Zotten oder der Muscularis mucosa einwirken. In einem solchen einfachsten Falle würde schon eine einfache multipolare Zelle einen vollständigen Reflexapparat darstellen."

Dogiel ('95) also described the neurones in the myenteric and the submucous plexuses in great detail. So successful was this investigator with his own methylene blue method that the accuracy and detail of his figures has scarcely been equaled by the work of later investigators.

Among the more recent investigators, Müller ('11) has given us a more or less detailed description of the myenteric and the submucous plexuses in various regions of the digestive tube. His figures and microphotographs indicate a wide range of variation among the neurones in these plexuses. His studies show, furthermore, that with some differences in detail the myenteric and the submucous plexuses are similarly constructed throughout the length of the digestive tube.

In spite of our knowledge of the general morphology of the sympathetic nervous system and the histological characters of its component neurones, little is known concerning the physiological relationships of the sympathetic neurones and the distribution of their axones and dendrites, especially in the peripheral sympathetic plexuses. The present paper is primarily an attempt to point out some of the morphological relationships of the neurones in the myenteric and the submucous plexuses to each other and to the muscle, gland and epithelial cells of the organs innervated by them; thus to advance our knowledge of the morphological basis for the physiological activities of the sympathetic nervous mechanism in the walls of the digestive tube.

I desire to express my indebtedness to Prof. G. L. Houser for suggestions during the progress of this investigation and for reading the manuscript. I am also indebted to Prof. F. A. Stromsten for suggestions in technique.

MATERIAL AND METHODS

The present series of observations is based primarily on preparations of the stomach and the small intestine of the cat and the dog. Good preparations for the study of the myenteric and the submucous plexuses are not easily obtained. The silver reduction method of Cajal and the method of Bielschowsky, both of which were employed more or less successfully for the

study of other parts of the sympathetic nervous system, failed utterly in the hands of the writer when applied to the digestive tube of the cat and the dog. After numerous unsuccessful attempts at intra-vitam staining with methylene blue, preparations of the stomach and the small intestine of the cat were obtained by the use of this method in which some of the neurones in the myenteric plexus and many of the fiber-tracts involving both the myenteric and the submucous plexuses were well stained and could be studied quite satisfactorily. Methylene blue preparations in which the cell-bodies of neurones in the submucous plexus were well stained were not secured.

The pyridine-silver method as employed by Ranson¹ was found very useful for the study of the neurones in both the myenteric and the submucous plexuses. In sections of the small intestine of the dog successfully prepared by this method, sympathetic neurones and fibers are well stained and may be satisfactorily studied. In these preparations, however, it is rarely possible to trace sympathetic fibers to their terminations on gland or epithelial cells. Even this method does not yield as uniformly good results when applied to the sympathetic plexuses in the walls of the digestive tube as when applied to other parts of the sympathetic nervous system or to the cerebro-spinal nervous system.

OBSERVATIONS

Myenteric plexus

The ganglia of the myenteric plexus are somewhat irregular flattened or less-shaped aggregates of neurones interposed between the longitudinal and the circular muscle-layers of the digestive tube. These ganglia are variously connected with each other by commissures of non-medullated fibers arranged either in distinct bundles or in broad flattened bands. In sections of the stomach or the small intestine taken in the plane of the myenteric plexus, these commissures may be traced from one ganglion into another. In many instances four or more commissures may be traced in as many directions from a single

¹ Amer. Jour. Anat., vol. 12. p. 69.

ganglion. Within the ganglia the paths of the fibers composing these commissures intersect each other at various angles, while in the commissures the fibers run more or less parallel with each other. In pyridine-silver preparations the slender fibers running in these commissures are stained somewhat more intensely than the surrounding tissue and present a slightly wavy and varicose appearance. In those commissures which are composed of a distinct fiber-bundle the fibers are more or less compactly aggregated, while in the more flattened commissures fibers running parallel with each other may often be observed distributed more or less uniformly over areas of considerable width. In transverse or longitudinal sections numerous commissures may be traced between the bundles of circular muscles from the ganglia of the myenteric plexus into the submucous plexus.

The ganglia of the myenteric plexus vary greatly in size, being composed of relatively few or of relatively many neurones. The neurones are not compactly aggregated. In good pyridine-silver preparations, however, little tissue may be observed in the ganglia except the fibers which pass through them at various angles and the neurones with their processes. Pericellular capsules were not observed in these ganglia either in pyridine-silver or in methylene blue preparations. The writer is, therefore, inclined to agree with Müller ('11) that such capsules do not occur in this plexus.

The neurones in these ganglia may be studied most satisfactorily in sections taken in the plane of the plexus because many of their processes lie approximately in this plane. In good preparations of this kind axones and dendrites may often be traced for a considerable distance from the cell-body. The neurones in these ganglia vary greatly in size as well as in their general character and form. Neurones of distinct types may be observed, but the deviations from such types are so numerous and so varied that one finds little satisfaction in attempting any rigid classification. Perhaps the most significant morphological difference between neurones in these ganglia consists in the length and the distribution of their dendrites. The dendrites of some are long and slender while those of others are too short to be traced out of

the ganglion in which the cell-body is located. These neurones have been so well described and illustrated by earlier observers that any attempt to illustrate their diversity of form in this paper would be superfluous. A few representative neurones taken from the ganglia of the myenteric plexus in the small intestine are illustrated in the accompanying figure (fig. 1, A, B, C).



Fig. 1 Sympathetic neurones. A, in myenteric plexus, ileum of cat; B and C, in myenteric plexus, ileum of dog; D, E, F, in submucous plexus, ileum of dog. *a*, axone. Spencer, obj. 1.8, oc. 8.

The axone is usually a slender, somewhat varicose fiber which arises either from the base of a protoplasmic process or directly from the cell-body. Not infrequently axones may be traced from their origin into the commissures connecting the ganglia of the myenteric plexus or into those leading from this plexus into the submucous plexus. In some instances axones may be traced

directly into the muscle-layers where they terminate on smooth muscle-fibers. Dendrites may also be traced from their cells of origin into the commissures. Although in many instances it is impossible to distinguish between axone and dendrites, there can be no doubt that dendrites are present in the commissures. Not infrequently several processes arising from the same cell may be traced into a commissure leading from the ganglion in one direction while other processes arising from the same cell may be traced into commissures leading from the ganglion in other directions. In transverse or longitudinal sections axones as well as many of the longer dendrites may be traced directly into the commissures leading from the myenteric plexus into the submucous plexus. In figure 2, A, which is taken from a longitudinal section of the ileum of a kitten prepared by the methylene blue method, is illustrated a commissure connecting a ganglion of the myenteric plexus with a ganglion of the submucous plexus. This commissure lies in the connective tissue between the muscle-bundles. Individual nerve-fibers can not be traced from one ganglion into the other in this section. However, nerve-fibers are present in the commissure throughout its entire length. Figure 2, B, is taken from a section through the posterior region of the oesophagus of an embryo of the chick of ten days incubation prepared by the pyridine-silver method. Neurones in the myenteric and the submucous plexuses do not appear in these preparations. Individual nerve-fibers may, however, be traced from the myenteric plexus to the level of the submucous plexus. There can be no doubt, therefore, that individual nerve-fibers extend from one of these plexuses into the other.

Submucous plexus

The ganglia of the submucous plexus, like those of the myenteric plexus, are variously connected by commissures of non-medullated fibers in which both axones and dendrites may be traced. These commissures, in which the fibers are usually more or less compactly aggregated, with the ganglia interposed at their nodal points form a network which in general is confined to the sub-

mucous layer. As indicated above, the submucous plexus is connected with the myenteric plexus by fibrous commissures. Fibers which have their origin in the ganglia of the submucous plexus may be traced into these commissures. Furthermore, nerve-fibers may be traced from the ganglia of the submucous plexus into proximity with the digestive glands where many of them terminate on gland-cells and into the gastric folds and

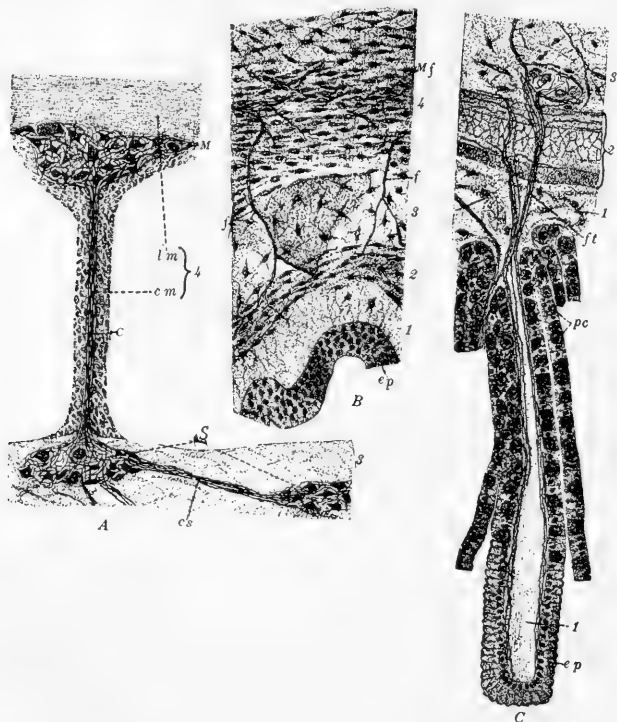


Fig. 2 A, from longitudinal section of small intestine of kitten, showing ganglia of myenteric and submucous plexuses with connecting commissures; B, from section through posterior region of oesophagus of ten day embryo of chick; C, from section through cardiac region of stomach of cat, showing nerve-fibers extending from submucous plexus into gastric fold. 1, tunica propria; 2, muscularis mucosae; 3, submucosa; 4, muscularis; c, commissure connecting ganglia of myenteric and submucous plexuses; cm, circular muscles; cs, commissure connecting ganglia of submucous plexus; ep, epithelium; f, nerve-fibers; ft, nerve-fibers extending from submucous plexus into gastric fold; lm, longitudinal muscles; M, ganglion of myenteric plexus; Mf, fibers in myenteric plexus; pc, parietal cells; S, ganglia of submucous plexus.

plicae and the intestinal villi where many of them terminate on cells of the digestive epithelium. Such fibers are illustrated in the accompanying figure (fig. 2, C, *ft*) which is taken from a section through the cardiac region of the stomach of the cat prepared by the methylene blue method.

The ganglia of the submucous plexus show a wider range in size and form than do the ganglia of the myenteric plexus. The latter, being interposed between the longitudinal and the circular muscle-layers, are more or less regular in form. The ganglia of the submucous plexus, on the other hand, are surrounded by loose connective tissue which apparently exercises little influence in determining their form. Some of these ganglia appear as small rounded or elongated cell-groups. Others are more or less irregular in form or even T- or Y-shaped according to the angles between the commissures which radiate from them. These ganglia may be relatively small, containing relatively few neurones, or relatively large, containing relatively many neurones. In all of them, however, the neurones are more compactly aggregated than are the neurones in the ganglia of the myenteric plexus. It becomes correspondingly more difficult, therefore, to trace out the processes of these cells. In sections of material successfully prepared by the pyridine-silver method taken in the plane of the plexus, however, this may be done quite satisfactorily. In good preparations of this kind little tissue appears in these ganglia except the fibers which pass through them at various angles and the neurones with their processes. In these ganglia also, as Müller ('11) has suggested, pericellular capsules are probably not present.

The neurones in the ganglia of the submucous plexus present quite as wide a range of variation in size and form as do the neurones in the ganglia of the myenteric plexus. They possess certain distinctive characters, however, by which the experienced observer may without difficulty recognize them as belonging to the submucous plexus. They are also relatively smaller than the neurones in the myenteric plexus. A few of these neurones taken from pyridine-silver preparations of the small intestine of the dog are illustrated in figure 1, D, E, F.

The great majority of the neurones in the ganglia of the submucous plexus are more or less regular in outline and possess relatively few dendrites which are usually long and slender and give rise to relatively few slender branches. The slender processes of these neurones may frequently be traced into the commissures connecting the ganglia of the submucous plexus, into the commissures connecting this plexus with the myenteric plexus or into the fiber-tracts leading from the ganglia of the submucous plexus into proximity with the digestive glands and into the gastric folds and plicae or the intestinal villi.

Fiber-terminations

In sections of the stomach and the small intestine of both the cat and the dog prepared by either the methylene blue or the pyridine-silver method, nerve-fibers may not infrequently be traced from the ganglia of the myenteric plexus quite directly into the longitudinal or the circular muscle-layer. Although individual fibers could not be traced from their origin to their termination on smooth muscle-cells, terminations of nerve-fibers on muscle-cells could frequently be observed. In good methylene blue preparations, slender varicose fibers may be observed in the inter-cellular cement between the muscle-cells. The terminal portions of these fibers are usually exceedingly varicose and frequently give rise to very slender lateral branches which terminate in minute terminal enlargements on the same muscle-cell on which the terminal portion itself ends or on another muscle-cell lying parallel with it. Figure 3, A, illustrates the terminal portion of a nerve-fiber with several lateral branches ending on the same muscle cell. Terminations of sympathetic nerve-fibers on smooth muscle-cells have been similarly described by Erik Müller ('92), Retzius ('92) and Huber ('97).

As indicated in an earlier section of this paper, sympathetic nerve-fibers may be traced from the ganglia of the submucous plexus into proximity with the digestive glands and into the gastric folds and plicae and the intestinal villi. In methylene blue preparations of the stomach of the cat, fibers could not infrequently be observed terminating on parietal cells (fig. 3, D).

In similar preparations of the small intestine sympathetic fibers may be traced into close proximity with the digestive glands and not infrequently such fibers could be observed among the gland-cells. There can be little doubt, therefore, that some of these fibers form physiological contact with cells of the digestive glands.

Many of the sympathetic fibers reaching down from the sub-mucous plexus may be traced beyond the level of the digestive glands into the gastric folds and plicae or the intestinal villi where many of them terminate on cells of the digestive epithelium.

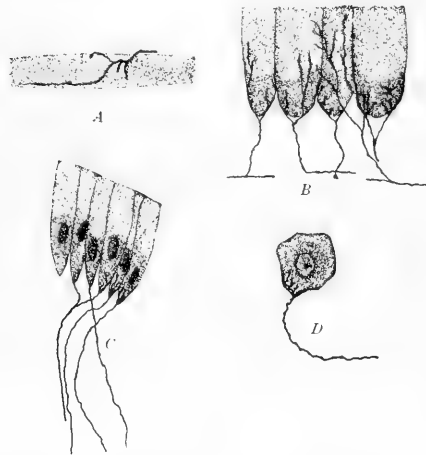


Fig. 3 Terminations of sympathetic nerve-fibers. A, on smooth muscle-cell; B, on cells of digestive epithelium, ileum of cat; C, on cells of digestive epithelium, stomach of cat; D, on parietal cell, stomach of cat. A and B, Leitz, obj. 1/16, oc. 4; C and D, Leitz, obj. 1/12, oc. 4.

These terminations are very delicate, but in good methylene blue preparations they may be observed without difficulty under high magnification. In methylene blue preparations of the stomach and the small intestine of the cat, terminations of this kind could not infrequently be observed on several adjacent epithelial cells. The sympathetic fibers extending into the gastric folds and plicae and the intestinal villi lie in the tunica propria close to the digestive epithelium. The terminal portions of these fibers or lateral branches given off by them deviate from the course of the fiber-

tracts toward the epithelial cells. On approaching the basal part of an epithelial cell the fiber breaks up into two or more branches which continue along the surface of the cell, giving off short branches along their course. These terminal branches may in some instances be traced along the surface of an epithelial cell for nearly half its length. Figure 3, B, illustrates the terminations of sympathetic nerve-fibers on epithelial cells in the ileum of the cat. In this instance the epithelial cells were but slightly stained.

The fibers terminating on them could, therefore, be traced to their distal extremities. Figure 3, C, illustrates similar sympathetic fiber-terminations on epithelial cells located at the free end of a gastric fold in the cardiac region of the stomach of the cat. In this instance the epithelial cells were stained more intensely. Consequently, the terminal portions of the fibers could be traced but for a short distance on their surfaces.

Whether the sympathetic nerve-fibers terminating on cells of the digestive epithelium are axones or dendrites can not be determined by their microscopic appearance. The fact that they terminate on epithelial cells, however, seems to warrant the conclusion that they are receptive fibers. Furthermore, in view of the preponderance in the ganglia of the submucous plexus of neurones with long slender dendrites, it is highly probable that they are the dendrites of these neurones.

Dogiel ('96) was led to believe, by observations of his pupil, Sakusseff, on the digestive tube of fishes, that sympathetic nerve-fibers actually terminate on cells of the digestive epithelium. The opinion, based on both anatomical and physiological considerations, that sympathetic nerve-fibers terminate on epithelial cells in the digestive tube has been repeatedly expressed by recent investigators. As far as the writer is aware, however, terminations of sympathetic nerve-fibers on cells of the digestive epithelium in higher vertebrates have not previously been described.

In both the myenteric and the submucous plexus, terminations of sympathetic nerve-fibers on sympathetic neurones may occasionally be observed. In methylene blue preparations such fiber-terminations may be studied most satisfactorily on neurones which are stained but lightly. Terminations of nerve-fibers on

neurones which are well stained may readily be overlooked because there is little difference in the intensity of the stain of the fiber and the cell on which it terminates. In the myenteric plexus in the small intestine of the kitten prepared by the methylene blue method, terminal nets could be observed on many of the smaller neurones which were stained so lightly that none of their protoplasmic processes could be traced and the cell-bodies appeared only in faint outline. A terminal net of this type is illustrated in figure 4, A. As the fiber approaches the cell-body on which the terminal net is formed it breaks up into two or more branches which twine about the cell, giving off numerous smaller branches along their course. These slender fibers interlace with

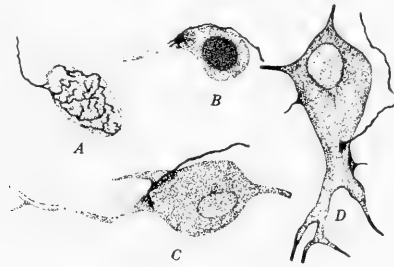


Fig. 4 Terminations of sympathetic nerve fibers on sympathetic neurones. Spencer, obj. 1.8, oc. 8.

each other until a network is formed which more or less completely encloses the cell-body. Terminations of this type have been repeatedly described in various parts of the sympathetic nervous system.

Another type of sympathetic fiber-termination on a sympathetic neurone which occurs also in the myenteric plexus is illustrated in figure 4, B. In this instance the fiber terminates, not on the cell-body, but on the proximal part of a large protoplasmic process in a flattened terminal enlargement from which several small protoplasmic processes reach out like slender pseudopodia. Fibers terminating on the proximal portions of large dendrites were observed also in the submucous plexus in pyridine-silver prepara-

tions of the ileum of the dog (fig. 4, C). In these instances, however, no distinct terminal enlargement is apparent, but the terminal part of the fiber breaks up into short delicate branches which spread out over the surface of the proximal part of the dendrite. Sympathetic fiber-terminations similar to those here described were described by Huber ('97) in other parts of the sympathetic nervous system.

In the myenteric plexus of the ileum of the dog prepared by the pyridine-silver method, sympathetic fibers were observed which terminate directly on the cell-bodies of large neurones (fig. 4, D). A sympathetic fiber terminating in this manner may without difficulty be distinguished from the processes which arise from the neurone because it is more slender than the latter and stains more intensely. Furthermore, the terminal enlargement can not be confused with the cone of origin of any of the protoplasmic processes. Terminations of sympathetic nerve-fibers on sympathetic neurones similar to those here described were described by von Lenhossék ('94) in Golgi preparations of embryos of the chick.

Whether the sympathetic fibers whose terminations on sympathetic neurones in the myenteric and the submucous plexuses are here described are the axones of neurones within these plexuses or whether they are fibers whose origin is in some center outside the walls of the digestive tube could not be determined. That some of them may represent fibers which arise in more centrally located centers is highly probable. On the other hand, it is highly probable that axones of neurones in either the myenteric or the submucous plexus may terminate on neurones in the same or in the other of these plexuses.

DISCUSSION

According to the doctrine of Langley which is still more or less prevalent, the neurones composing the sympathetic ganglia are all excitatory in character. These neurones, whether located in the ganglia of the sympathetic trunks, the prevertebral or the peripheral sympathetic plexuses, are interpreted as links in effer-

ent chains. According to this doctrine, the only sensory neurones associated with the sympathetic nervous system are the visceral afferent neurones whose cell-bodies are located in the cerebro-spinal ganglia. According to this scheme, all sympathetic reflexes involving sensory neurones must involve an entire afferent and an entire efferent chain. Local reflexes involving only sympathetic elements, if they occur at all, can occur only as axone-reflexes involving only excitatory neurones.

This conception of Langley regarding the physiological relationships of the sympathetic neurones is based largely on the results of experimental methods which are, doubtless, better adapted to determine the constitution of the visceral afferent and the visceral efferent chains than to determine whether or not local reflexes involving sensory and motor elements occur in the peripheral sympathetic plexuses.

The facts presented in this paper strongly suggest that the sympathetic system, like the other functional divisions of the nervous system, is essentially a system of reflex arcs, involving both sensory and motor neurones, some of which are strictly local in character, while others are less local or even involve centers in the cerebro-spinal nervous system.

That local reflexes occur in the peripheral sympathetic plexuses is shown by experimental observations. Recent experimental investigations, notably those of Bayliss and Starling ('99), Cannon ('06) and Åuer ('10), have shown conclusively that the motor activities of the digestive tube may be carried on more or less normally for a considerable period after the nerves connecting the digestive tube with the cerebro-spinal nervous system have been severed. The possibility that axone-reflexes may occur under such circumstances is not precluded. Furthermore, it is well known that the effect of adrenalin on involuntary muscles is the same as the effect of sympathetic stimulation. Nevertheless; in view of the fact that, as shown in this paper, some of the neurones in the submucous plexus send their dendrites into the gastric folds and plicae and the intestinal villi where many of them terminate on cells of the digestive epithelium, it is highly probable that these neurones are stimulated either directly or indirectly by the

presence of food in the digestive tube and that their impulses are transmitted to motor neurones with which they form physiological contact. The axones of some of these 'receptive' neurones in the submucous plexus may be traced into the commissures connecting the ganglia of the submucous plexus or into those connecting this plexus with the myenteric plexus. Furthermore, dendrites as well as axones extend from the myenteric plexus into or through the submucous plexus. The anatomical relationships of the sympathetic neurones in the myenteric and the submucous plexuses, doubtless, are such as to provide both shorter and longer reflex arcs involving both sensory and motor neurones. We may, therefore, conceive of sympathetic reflexes which are strictly local in character while others pass through several or even many ganglia and thus transmit impulses from one level of the digestive tube to another separated from it by an appreciable interval. Still other reflexes stimulated in the same manner may involve centers in the prevertebral sympathetic plexuses or in the sympathetic trunks. Besides these types of reflexes, doubtless, sympathetic reflexes occur which involve centers in the spinal cord and the brain. The fact that the motor activities of the digestive tube may be carried on more or less normally when the paths for these longer reflexes are severed, however, seems to indicate that the normal nervous control of the digestive functions is exercised primarily by the local sympathetic mechanism.

The schematic diagram in the accompanying figure (fig. 5) is introduced to illustrate some of the probable relationships of the sympathetic neurones in the myenteric and the submucous plexuses. The motor neurones in the diagram are stippled while those which are supposedly sensory appear solid.

That all the types of reflexes described above actually occur in the sympathetic nervous system has not been demonstrated experimentally. As has been pointed out, however, the orientation of the sympathetic neurones in the myenteric and the submucous plexuses and the peripheral distribution of their axones and dendrites is obviously such as would be required by a system of shorter and longer reflex arcs. Therefore, the conclusion that all the types of sympathetic reflexes above described are possible

in the nervous control of the digestive functions can hardly be avoided.

The nervous mechanism in the walls of the digestive tube is connected with the cerebro-spinal nervous system primarily by the vagi and the splanchnics. In general the vagi act in an excit-

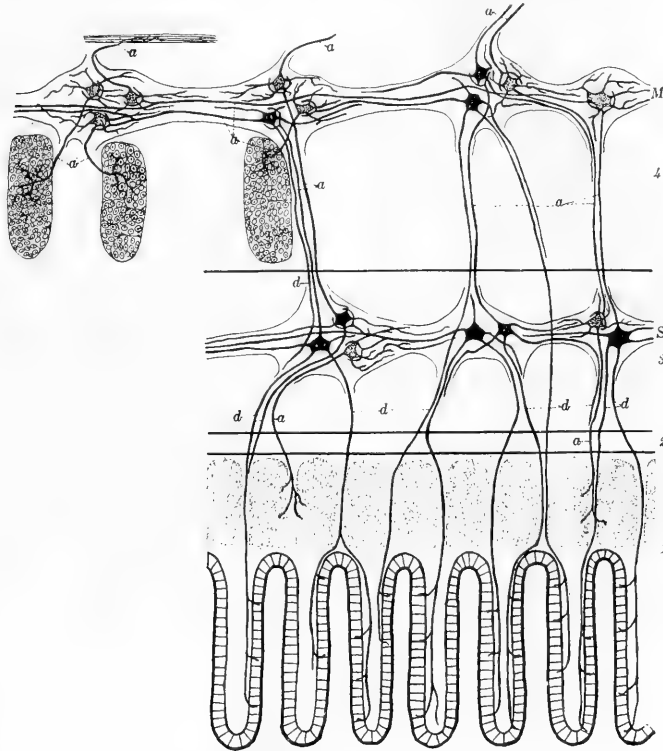


Fig. 5 Schematic diagram illustrating probable relationships of sympathetic neurones in myenteric and submucous plexuses. Motor neurones, stippled; sensory neurones, solid. 1, tunica propria; 2, muscularis mucosae; 3, submucosa; 4, muscularis; M, myenteric plexus; S, submucous plexus; a, axones; d, dendrites.

atory and the splanchnics in an inhibitory manner on the digestive organs. The recent work of Cannon ('06) and Äuer ('10) shows that the vagi possess both excitatory and inhibitory fibers for the digestive tube. The work of these investigators shows, furthermore, that while the splanchnics are not necessary for the

quite normal control of the digestive functions in the presence of vagus influences, these functions are carried on more nearly normally with both the splanchnics and the vagi severed than with the splanchnics alone intact. These facts seem to indicate the fundamental importance of the vagi in the extrinsic nervous control of the digestive organs. This is suggested, also, by the facts of evolution. The writer has presented evidence in an earlier paper ('11) in support of the theory that the peripheral sympathetic plexuses which are genetically related to the vagi represent those parts of the sympathetic nervous system which arose earliest in the process of evolution and that the vagi constitute the primary connection between these plexuses and the cerebro-spinal nervous system. We should expect, therefore, that the vagi constitute also the primary functional connection between the nervous mechanism in the walls of the internal organs and the cerebro-spinal nervous system.

The normal nervous control of the digestive organs is, doubtless, exercised more or less directly by the local sympathetic mechanism, the general control which is normally exercised by extrinsic nerves being largely tonic in character. Inasmuch as the vagi form the primary connection between the cerebro-spinal nervous system and the sympathetic plexuses in the walls of the digestive organs, it is highly probable that the major part of such tonic control is exercised by the vagi.

SUMMARY

1. The ganglia of the myenteric plexus are interposed between the longitudinal and the circular muscle-layers of the digestive tube. The ganglia of the submucous plexus are imbedded in the submucous layer. The ganglia of each of these plexuses are variously connected by commissures of non-medullated fibers among which may be traced both axones and dendrites.

2. The myentric and the submucous plexuses are connected with each other by fibrous commissures. Nerve-fibers also extend from the submucous plexus into proximity with the digestive glands where many of them terminate on gland-cells and into the

gastric folds and plicae and the intestinal villi where many of them terminate on cells of the digestive epithelium. The fibers which terminate on cells of the digestive epithelium are, doubtless, the dendrites of 'receptive' or sensory neurones.

3. The orientation of the neurones in the ganglia of the myenteric and the submucous plexuses and the distribution of their axones and dendrites strongly suggest that the sympathetic system, like the other functional divisions of the nervous system, is essentially a system of reflex arcs involving both sensory and motor neurones, some of which are strictly local while others are less local or even involve centers in the cerebro-spinal nervous system.

4. The normal nervous control of the digestive functions is probably exercised primarily by the local sympathetic mechanism, the general control which is exercised by extrinsic nerves being largely tonic in character. The major portion of such tonic control is probably exercised by the vagi.

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THE CENTRAL NERVOUS SYSTEM IN A CASE OF CYCLOPIA IN HOMO

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University*

FIFTY-ONE FIGURES

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INTRODUCTION

Although cyclopia in man is a somewhat rare condition, yet among the cases reported few deal with other than the external features of the malformation. Up to the present, the only investigation into the finer structure of the central nervous system in a case of cyclopia in homo has been made by O. Naegeli in

v. Monakow's laboratory (17). In the case reported by him, however, the condition of cyclopia was much complicated by the presence of a very extensive malformation in connection with the brain stem and cord, which without doubt was quite independent of the cycloplan condition.

With the exception of a moderate degree of hydrocephalus, the present case presents no malformations other than those which may safely be considered as due to the cyclopic condition. Such being the case, this material is well adapted to determine among other things, whether the cycloplan brain can in any way be regarded as an arrest of development at an early phylogenetic stage, as Naegeli suggests. The research was begun in the hope of clearing up this point, and as will be shown, all the evidence in this case is against the assumption of such a reversion.

The central nervous system is of paramount interest in this case, and the necessarily incomplete general description is only warranted in so far as it records certain data which may be of use in any future complete investigations. In the course of the work, the condition of development in the pallium has proved to be of the greatest interest, as it tends to throw additional light on the subject of the evolution of the normal cortex. This phase of the research has not been as fully dealt with here as is desired, and will be the subject of a future communication.

The cycloplan foetus, upon which the following observations have been made, was obtained in Chicago through the courtesy of Dr. Warren H. Hunter, Cook County Coroner's Physician, and Prof. E. R. Le Count of Rush Medical College, Chicago. The clinical details of the case have already been reported by Dr. Harry Jackson (11).

I am indebted to Prof. C. J. Herriek for the opportunity of taking up this work and for much helpful criticism throughout the investigation. The work was begun in the Anatomical Department of the University of Chicago in the spring quarter of 1910. The major portion of the technique in connection with the microscopic study of the central nervous system and also the examination of this material has been done in the Anatomical Department of Western Reserve University.

The specimen was preserved immediately after death, under Dr. Le Count's direction, by an injection through the carotid artery of 10 per cent formalin (4 per cent formaldehyde) after which the whole body was immersed in a solution of the same. On account of this careful fixation, the histological preservation was excellent.

A research had been begun upon this specimen before it came into my possession, and on this account I have been unable to determine the exact attachments of the cerebral roof over the thalamic mass. I am indebted to Miss Katherine Hill, medical artist at the Hull Laboratory of Anatomy, for the four drawings of the entire brain made shortly after its removal from the skull cavity and before the relations referred to above had been disturbed.

GENERAL DESCRIPTION OF THE CASE

External features

The body is that of a well developed male infant weighing seven and one-half pounds. The head is enlarged and shows a condition of moderate hydrocephalus so that the cranium cerebrale is considerably more developed than the cranium viscerale. The skull is dolichocephalic, though not markedly so, as is usually the case in hydrocephalus (18). It measures 95 mm. in greatest transverse diameter, and 130 mm. in greatest longitudinal diameter, the cephalic index being 73. The sagittal suture is widely open, the parietal bones being separated from one another by a considerable space (2 to 3 cm.) throughout. The metopic suture is also widely open, its lateral margins being separated in the upper part by a distance of 10 mm.

At the base of the regio frontalis, which is high and prominent, in the mid-line is a finger-like process of about 2 cm. in length. The walls are firm and it presents at its distal extremity a single orifice which leads into a blind passage extending inward as far as the attached end of the organ. This appendage, which probably represents an abortive naso-frontal process, overhangs the single median eye (fig. 1).

The eyeball protrudes from the orbital fossa and is somewhat larger in its transverse than in its vertical diameter. The cornea is 'dumb-bell' in shape—the long axis of the dumb-bell, which is slightly asymmetrical, being transverse. The condition of the pupil could not be accurately determined on account of the opacity of the cornea. The exposed superior surface of the bulb is of dark brownish black color, due to the thinness of the



Fig. 1 Photograph of the specimen

sclera overlying the uveal pigment. The superior palpebrae are represented by two thickened ridges sparsely beset with cilia and separated from one another by a notch immediately below the base of the overhanging proboscis. In either side a notch representing the outer canthus limits these ridges laterally. The inferior palpebrae are represented on either side by short blunt tubercles bearing a few cilia and situated immediately below

and to the inner side of the external canthi. The remainder of the inferior margin of the orbit for the short distance medial to the tubercles representing the lower lids is formed on either side by the palatine processes of the maxillary elements. Owing to a condition of hare lip and partial cleft palate, a boundary in the mid-line ventrally is lacking between the mouth cavity and the conjunctival sac above.

It thus happens that the mucous membrane lining the roof of the mouth becomes continuous with that lining the conjunctival sac, around the margins of the cleft palate (fig. 2). The

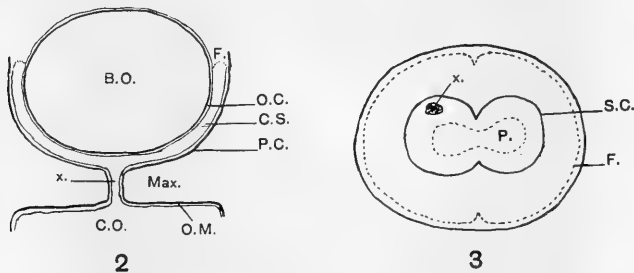


Fig. 2 Schematic coronal section through face region, showing relations of oral cavity and conjunctival sac. *B.O.*, bulbus oculi; *C.O.*, oral cavity; *O.C.*, ocular conjunctiva, continuous with the parietal layer (*P.C.*), at the fornix conjunctivae (*F.*), which is indicated in dotted lines as being on a deeper plane; *O.M.*, oral mucosa which becomes continuous with the parietal layer of the conjunctiva (*P.C.*), through the cleft (*X.*) between the maxillary elements (*Max.*).

Fig. 3 Diagram of anterior surface of bulbus. *F.*, fornix conjunctivae indicated in dotted lines; *P.*, pupil; *S.C.*, sclero-corneal junction; *x.*, small pigmented area at margin of cornea.

condition of cleft palate ceases about 3 cm. from the red margin of the upper lip. In the median line at the point of closure of the palatine cleft the parietal layer of the conjunctiva becomes reflected on to the eyeball. On both sides lateral to this point of attachment, the conjunctival sac extends for some distance backward before the inferior fornix is reached. A similar condition obtains above, the two lateral pouches being separated from one another in the mid line dorsally at the notch situated at the base of the proboscis. These relations will be made clear by reference to the diagram (fig. 3), where the line of reflection of the bulbar conjunctiva is indicated in dotted lines.

There is thus every indication of bilateral symmetry in the cornea, the conjunctival sac, the palpebrae, and, as will be subsequently seen, in the contents of the orbital fossa.

The uvula and soft palate are present but the posterior nares are represented only by a slight recess above these structures.

Cranial cavity, dura in situ

As it was desired to preserve the external form of the specimen for museum purposes, many of the following descriptions are necessarily incomplete.

The cranial cavity presents a somewhat elongated appearance, as is indicated in figure 4, and is divided into anterior, middle and posterior fossae.

Posterior fossa. The posterior fossa and its boundaries are approximately normal in appearance. The dural foramina for the exit of the nerves normally leaving the cranial cavity in this region may be made out with the exception of the foramen for the trochlear nerve. The vertebral arteries and the accessory nerves may be made out in the foramen magnum. The internal occipital protuberance is well marked. The attachment and extent of the tentorium are normal.

Middle fossa. The middle fossa is subdivided into two lateral portions by a much elongated, sharp, median ridge. Slightly more than 1 cm. from the anterior end of this ridge, a single large artery opens on its dorsal surface. At its anterior extremity the median ridge becomes continuous with the median process of the posterior boundary of the anterior fossa.

Anterior fossa. The anterior fossa, which is quite extensive, is bounded on either side posteriorly by curved ridges whose concavity is directed backward.

Cranial cavity, dura removed

Anterior fossa. No ethmoid element can be made out. A wide metopic suture is present (fig. 5). In the mid-line, slightly in front of the junction of the posterior boundary of the anterior fossa with the median ridge of the middle fossa, there is seen a slight prolongation of the dura into a small pit in the bone.

This subsequently was found to mark the point of attachment of the fibrous remnants of the optic nerves.

Middle fossa. The two carotid arteries enter the skull on either side of the median ridge postero-laterally. The left carotid is a mere fibrous thread, while the right carotid is somewhat larger than normal. The left carotid canal is proportionately reduced. These vessels approach one another, become united on the crest of the median ridge and pass forward as a single vessel. At the

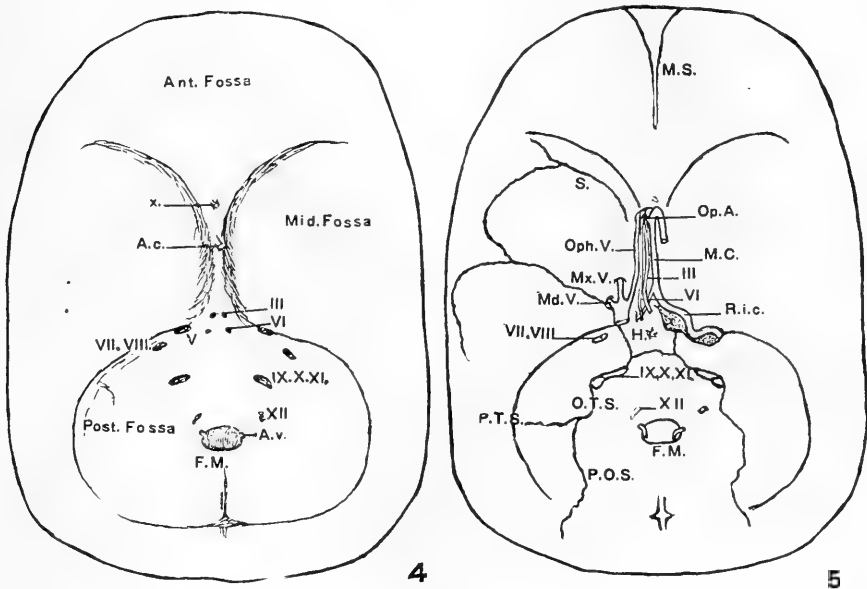


Fig. 4 Diagram of floor of skull cavity, dura in situ. *A.c.*, carotid artery *A.v.*, vertebral artery; *F.M.*, foramen magnum; *x.*, a slight depression in dura which marks the site of attachment below of the fibrous remnant of the optic nerve. The cranial nerves in their dural foramina are numbered in Roman numerals.

Fig. 5 Diagram of floor of skull cavity, dura removed. *F.M.*, foramen magnum; *H.*, pit in basi-sphenoid into which were prolonged fine processes from dura; *M.C.*, median carotid artery; *M.S.*, metopic suture; *O.p.A.*, ophthalmic artery; *O.T.S.*, occipito-temporal suture; *P.O.S.*, parieto-occipital suture; *P.T.S.*, parieto-temporal suture; *R.i.c.*, right internal carotid artery; *S.*, suture between great wing of sphenoid and frontal element; *X.*, marks the cut surface of bone removed to expose the course of right internal carotid artery; cranial nerves indicated in Roman numerals. The remnant of the left internal carotid artery is indicated beneath the cut ends of the third and sixth nerves.

anterior extremity of the ridge a small vessel is given off and passes downwards through a median foramen to the orbit. This vessel is an azygos ophthalmic artery. Immediately after the ophthalmic branch is given off, the main vessel turns backward upon itself and courses a short distance in this direction before passing upward through the dura as the single median artery already noted. There is no appearance whatever of anterior or posterior clinoid processes.

On either side of the median ridge the third and sixth nerves pass forward beneath the dura to the median foramen mentioned above to gain the orbit. Slightly more laterad the ophthalmic division of the fifth nerve passes forward to the same destination. Accompanying the latter is a small nerve which may (?) represent the trochlear though subsequent dissection failed to identify it definitely.

The speno-temporal suture may be clearly made out, extending outward from the region of the carotid canal. Anteriorly another suture may be made out passing parallel to the posterior boundary of the anterior fossa, and marking the line of union between the frontal element and the great wing of the sphenoid.

Below the posterior end of the median crest, between the entering carotid arteries and behind the point of their junction, there is found a small pit in the basi-sphenoid into which are prolonged a few fine processes from the dura. This depression was not apparent before removal of the dura. Further examination showed that the fornix pharyngis was situated immediately below this pocket. In fact it marks the site where normally the pituitary body should be lodged. Although no pituitary tissue could be identified, I am inclined to view this pit as a remnant of Rathke's pouch.

Posterior fossa. In the posterior fossa the occipito-temporal, the parieto-occipital and the parieto-temporal sutures can readily be made out. The foramina for the exit of the nerves in this region presented no peculiarities.

Vascular anomalies

As has been already noted, the left internal carotid artery within the skull was reduced to a mere thread-like vessel. Examination also showed that the left carotid canal was proportionately reduced. The right internal carotid is somewhat larger than normal, as might be expected. In examining the origin of these vessels in the neck region it was found that the relations on the right side were normal. On the left side the internal carotid was so much reduced as to be distinguished only with difficulty. It arises from the posterior aspect of the common carotid at about the level of origin of the lingual artery. The left facial artery is somewhat larger than the right (diagram, fig. 6).

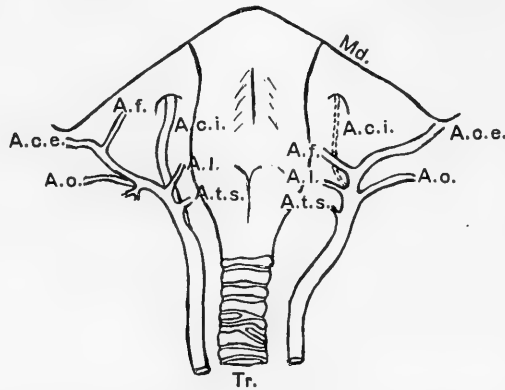


Fig. 6 Diagram of arrangement of arteries in neck region. *A.c.e.*, external carotid artery; *A.c.i.*, internal carotid artery; *A.f.*, facial artery; *A.l.*, lingual artery; *A.o.*, occipital artery; *A.t.s.*, superior thyroid artery; *Md.*, lower jaw; *Tr.*, trachea.

The course of the internal carotid arteries within the skull to the point where a single median vessel pierces the dura, has already been described.

Encephalic arteries. The vessels of this region are represented in figure 7 as pinned out and seen from above. Posteriorly the two vertebral arteries unite in a normal fashion to form the main basilar trunk. The origin of the anterior spinal artery is normal. The posterior inferior cerebellar arteries are small and arise from the basilar. A similar anomaly has been reported by

Blackburn (1) in a few of his cases. The basilar artery is long and gives off numerous irregular branches. Six pairs of these branches arise in the pontine region. The superior and anterior inferior cerebellar arteries arise from a common branch of the basilar. Of the two, the superior cerebellar is much the smaller.

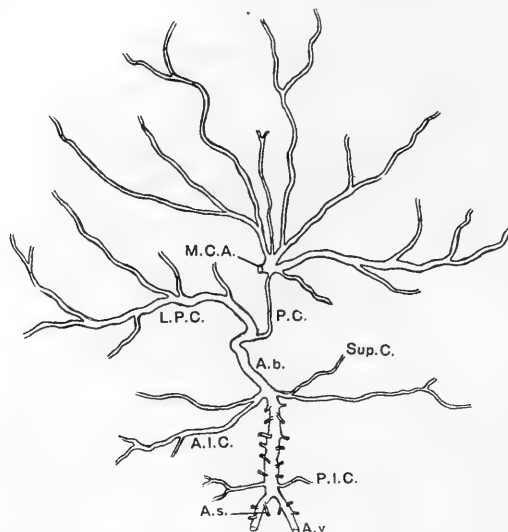


Fig. 7 Diagram of encephalic arteries pinned out and seen from above. *A.b.*, basilar artery; *A.I.C.*, anterior inferior cerebellar artery; *A.s.*, spinal artery; *A.v.*, vertebral artery; *L.P.C.*, left posterior cerebral artery; *M.C.A.*, median carotid artery; *P.C.*, posterior communicating artery; *P.I.C.*, posterior inferior cerebellar artery; *Sup.C.*, superior cerebellar artery. $\times \frac{1}{2}$.

Blackburn (q.v.) has noted that when the anterior inferior cerebellar artery is ill developed, the superior cerebellar artery occasionally sends branches to reinforce it. He also noted that when the posterior inferior cerebellar arteries are absent or small, and arising from the basilar, the anterior inferior cerebellar arteries send down compensating branches. In this case, both superior and posterior inferior cerebellar arteries are small and the anterior inferior cerebellar arteries are proportionately enlarged and send branches in both directions.

Anteriorly the basilar artery becomes continuous with a large left posterior cerebral vessel. No right posterior cerebral artery is present. A small median branch passes forward at the point of origin of the left posterior cerebral artery. This vessel is to be considered as a single posterior communicating artery. It is represented in figure 50 as being much larger than is actually the case.

From the median carotid trunk the cerebral vessels radiate out in such a manner that they become distributed to the right side and anterior portion of the left side of the cerebral vesicle. The posterior portion of the cerebral vesicle on the left side is supplied wholly by the posterior cerebral branch of the basilar artery.

From the vertebral arteries posteriorly to the point of origin of the common trunk of the superior and anterior inferior cerebellar arteries, the encephalic vessels have shown but little variation from the normal condition and may be identified with a reasonable degree of certainty.

Beyond this point there has been a very marked disturbance in the origin and relations of the various vessels supplying the cerebral vesicle.

Mall (14) has shown that under normal circumstances the arrangement of the encephalic vessels arising as branches from the future circle of Willis varies remarkably at different stages in the growth of the embryo.

Anomalies of the encephalic vessels are very commonly met with among the insane (1), more so than among normal individuals. The above fact implies that the growth and arrangement of these vessels is influenced in no small degree by the growth of the cerebral tissue. Very slight anatomical variations in the latter increase the tendency toward vascular anomalies.

When, as in the case in point, there has been a profound disturbance in the development of the primary forebrain vesicle a correspondingly wide deviation from the normal arterial arrangement may be looked for. The anomalies will be partly due to the mechanical difficulties encountered in growth, but mostly to the absence of certain parts of the cerebral tissue itself.

Thus the conditions of these vessels may be looked upon as being to some extent an indication of the degree of perfection of cerebral growth. The two disturbances cannot be considered as being mutually dependent upon one another, for the cerebral condition in cases such as this is certainly the prime factor.

There is a large blood supply from the median carotid to the right half of the cerebral vesicle, while there is no component to this side from the basilar artery. On the other hand, the left side of the cerebral vesicle receives a large component from the basilar artery which effectually compensates for the small supply derived from the branches of the median carotid trunk on this side. So, although the vessels are asymmetrically arranged, it is to be noted that the blood supply is approximately equal on both sides.

The sharp bend which is seen anteriorly in the median carotid before it pierces the dura is similar to the bending of the normal bilaterally symmetrical vessels before their division into anterior and middle cerebral arteries. It is to be noted in this connection that the single ophthalmic artery arises at this 'genu' as is also the case with the normal ophthalmic arteries.

Contents of the orbital fossa

The following incomplete dissections were made by removing the roof of the orbital fossa. Only those structures which could be dissected without disturbing the external relations of the bulbus are described. The diagrams represent the structures in this region as seen from above. All the structures examined were essentially symmetrical.

First stratum (fig. 8). On removing the bony roof of the orbital fossa and dissecting away the peribulbar fat and connective tissue, the muscles of this region were found to radiate outwards to their insertions from a central fibrous mass in which are imbedded the remnants of the unpaired optic nerve.

In the region of the central tendon the third nerve divides into a number of small branches and freely communicates with its fellow of the opposite side.

The sixth nerve comes to lie ventral to the oculomotor and in the region of the central tendon turns laterad and passes to the inferior surface of a muscular band which, from its nerve supply and relations to the bulbus, represents the rectus lateralis muscle.

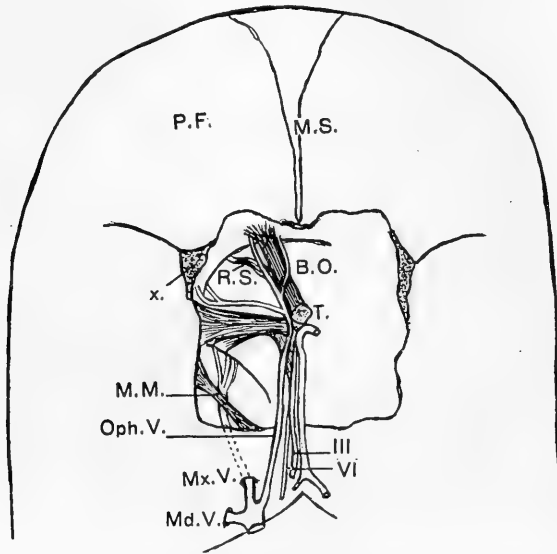


Fig. 8 Diagram of structures exposed on removing the bony roof of orbit. The metopic suture (*M.S.*) and an outline of the skull fossa are indicated, though not in proportion to the dissection of the orbit. *B.O.*, bulbus oculi; *Md.V.*, mandibular division of the fifth nerve; *M.M.*, Müller's muscle overlying the maxillary division of the fifth nerve (*Mx.V.*); *Oph.V.*, ophthalmic division of the fifth nerve; *P.F.*, squama frontalis; *R.S.*, rectus superior muscle; *T.*, central tendinous mass; *x.*, represents cut surface of bony ridge separating anterior and middle skull fossae; *III*, oculomotor nerve; *VI*, abducent nerve.

Lateral to the nerves mentioned, the ophthalmic division of the fifth nerve passes into the orbital fossa. Upon its dorso-medial surface there lies a small nerve which has already been alluded to as the possible representative of the trochlear. The relations of this nerve could not be accurately followed after reaching the region of the central tendon. Here it becomes lost in the median mass of fibrous tissue.

The ophthalmic division of the fifth nerve is the most dorsally placed nerve trunk in the orbital fossa and at the level of the central tendon it divides into two branches of about equal size. The most mesial branch divides into two parts upon the dorsal surface of a flat muscular band whose origin is from the common central tendon and whose insertion is into the deep fascia of the skin lateral to the notch representing the inner canthus. The branches of the nerve become related to the mesial and lateral borders of this muscle.

The more lateral branch courses laterad almost at right angles to the parent trunk upon the anterior border of a second flat muscular band arising from the central tendon and being inserted into the fascia in the region of the external canthus. All the branches of the ophthalmic division of the fifth nerve enter the subcutaneous tissue at the bony margin of the orbit. Although considerable disturbance occurred in this case in the areas usually supplied by the supratrochlear, frontal, and supraorbital nerves, it is possible that the branches of the fifth nerve here described may correspond to these.

The flattened bands of muscular tissue described in connection with the branches of the fifth nerve are present in essentially similar relations on both sides and probably represent an anomalous arrangement of the levator palpebrae superioris muscle.

Both foramen ovale and foramen rotundum, transmitting respectively the mandibular and maxillary divisions of the fifth nerve, are illustrated in figure 8, and the course of the maxillary division is indicated in dotted lines till it reaches the space opened for the dissection of the eye. The posterior boundary of the orbital fossa is here seen to be formed by two rounded bony eminences having their convex anterior borders inclined to one another in such a fashion as to form a V-shaped notch in the midline. Examination shows that these apparently represent the bony roofs of the tooth crypts. It is to be noted that the posterior boundary of the orbital fossa is a considerable distance behind the region of the central tendon.

The maxillary division of the fifth nerve passes over the roof of the crypt and is lost sight of at about the middle of the an-

terior convex margin where it dips down beneath the bulbus oculi. An anomalous band of muscular tissue passes obliquely across the upper aspect of the maxillary element and over the 2nd division of the fifth nerve. From its relation to the nerve this muscle may (?) represent the rudimentary bundle described as Muller's muscle in the normal orbital fossa.

Second stratum (fig. 9). The third and sixth nerves, and the ophthalmic division of the fifth nerve on each side, together with

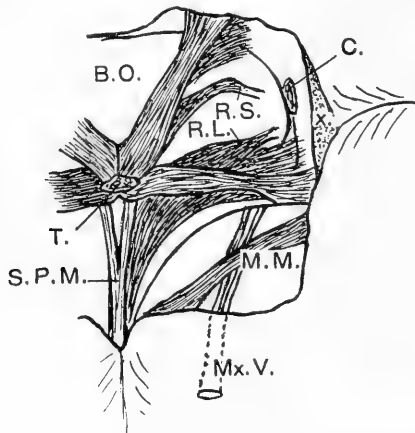


Fig. 9 Diagram of structures exposed in dissection of orbital fossa from above; second stratum. *C.*, small opening in fornix conjunctivae; *R.L.*, rectus lateralis muscle; *S.P.M.*, superior postero-median muscular band; other lettering as in figure 8.

the median carotid artery were cut posteriorly and reflected forward to expose the muscular mass situated beneath (they are not represented in the figure).

From the median notch formed by the junction of the two maxillary elements there arises a thin muscular band which anteriorly becomes divided into two symmetrical halves as indicated in the diagram. It becomes inserted into the central tendinous mass. On reflecting this muscle forwards, another similar, though slightly larger, muscular band is seen lying immediately beneath it. (For the sake of convenience in description these muscles will be termed the superior and inferior

postero-median muscular bands respectively). Its origin and insertion are similar to the first mentioned muscle and are shown in the next dissection.

Lateral to these muscular bands and situated on a lower plane, there is seen a broad sheet of muscular tissue whose fibers are more or less parallel to the anterior convex border of the maxillary element. The relations of this mass are more clearly brought out in the next dissection.

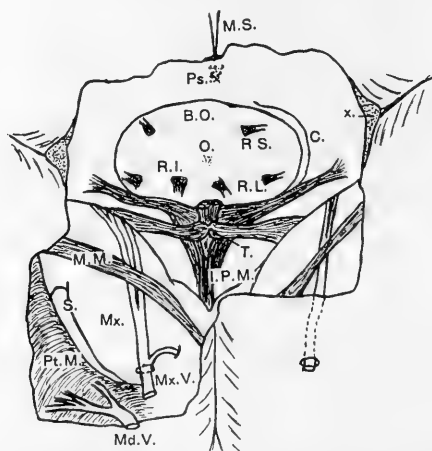


Fig. 10 Diagram of structures exposed in dissection of orbital fossa from above; third stratum. *I.P.M.*, inferior postero-median muscular band; *Mx.*, maxillary element; *Ps.*, point of attachment of proboscis; *Pt.M.*, muscular mass probably belonging to the pterygoid group; *R.I.*, rectus inferior muscle; *S.*, deep fossa behind maxillary element. Note the arrangement of the muscle *I.P.M.* and the one overlying it is such as to suggest the use of the term 'retractor bulbi.' Other lettering as in figure 8.

Third stratum (fig. 10). The entire muscular mass representing the levator palpebrae superioris and the superior portion of the postero-median muscular band were removed. The vessels and nerves were also removed with these. It was noted that a small branch of the ophthalmic artery entered the bulbus oculi in the mid-line at the lower margin of the fibrous optic nerve. With the exception of the rectus lateralis the muscular mass in relation to the eye received its innervation in a very irregular fashion from branches of the third nerve. The sixth nerve, as

has already been noted, passed directly to the inferior surface of the latter muscle.

On the left side, the opening in the bony roof of the orbit was continued posteriorly and the floor of the middle fossa of the skull was removed to the level of the foramen ovale. It will be here noted that the whole extent of the upper surface of the left maxillary element is exposed, together with the maxillary division of the fifth nerve and its overlying muscular band. Posteriorly is seen a well marked deep fossa. The mandibular division of the fifth nerve divides into a number of branches which pass into a thick muscular mass which, as far as it can be judged from its location, probably represents some part of the pterygoid group.

Posteriorly in the orbital fossa is seen the inferior portion of the postero-median muscular band. Also at this level from the region of the central fibrous mass two muscular bands pass laterad to be inserted into the fibrous tissue on the anterior surface of the bony wall of the maxillary element. At a lower level in this region is seen a layer of muscle whose fibers have already been noted as coursing laterad parallel to the anterior convex border of the crypt. It is seen to have a somewhat wide origin in the mid-line below the central tendinous mass. As the fibers pass outwards they converge and are inserted into the deep fascia at the lateral margin of the orbital fossa.

Two muscles, which from their relations represent the inferior recti, pass almost directly downwards from the central tendon to their insertions on the bulbus. The insertions of the rectus superior and lateralis muscles are also indicated.

In the mid-line in front, the roof of the orbit has been dissected away to show the point of attachment of the proboscis and its relation to the eye.

The arrangement of the orbital contents throughout is very similar to that obtaining in the cyclopiian eyes dissected by Wilder (28). As in his cases, the mesial recti muscles are absent owing to the complete suppression of the area in which they normally develop. The superior oblique muscle was not identified in this case.

MACROSCOPIC DESCRIPTION OF THE BRAIN

For the sake of convenience in description the following terms have been made use of. Those structures which together form that portion of the brain anterior to the pineal region are collectively spoken of as the primary forebrain vesicle. The primary forebrain vesicle is further subdivided into an anterior cerebral vesicle or cerebrum, and a posterior thalamus or thalamic mass.

Primary forebrain vesicle

From above: As seen in figure 48, this region appears as a large unpaired vesicle having a smooth arched roof which extends caudad to a point immediately in front of the corpus pineale. At no point does it arch over the posterior brain segments.

From the side: In the lateral line is here seen a very distinct sulcus passing in a circumferential manner around the cerebral vesicle. Its origin is hidden posteriorly in a deep fissure which exists between the basal portion of the primary forebrain vesicle and the brain stem. The relation of this sulcus may be seen by comparing figures 48, 49 and 50. It sharply marks off the smooth bulging roof from the thickened and somewhat furrowed base.

From below: The general configuration of the inferior surface of the primary forebrain vesicle is well illustrated in figure 50. It is seen to be divided by a quite marked Y-shaped furrow into two paired posterior lobes and a single azygos anterior lobe. This furrow cannot be compared with any sulci appearing on the surface of the normal cerebral hemisphere. It is apparently only the result of a mutual adaptation between the cerebral vesicle and the floor of the skull cavity. Smaller secondary furrows are seen on each of the lobes but the direction in each case is always at an angle to the main Y-shaped furrow. Apparently these very shallow sulci are due mainly to the presence of blood vessels. In the azygos anterior lobe a small fossa is seen directly in front of the diverging limbs of the Y-shaped principal furrow.

There is no appearance whatever of olfactory lobes, optic nerves or tract, infundibulum, or in fact of any structure normally appearing in a basal view of this portion of the brain.

As has been noted, the basal portion of the cerebrum, which posteriorly is more or less bilaterally symmetrical, is separated from the cerebellum by a very deep fossa.

From above on removal of the roof: On laying back the smooth, thin, arched roof, a cavity is brought to view which represents the dilated ventricular cavity of the primary forebrain. The floor of this cavity, which is quite vascular, is seen in figure 51 to be marked by a Y-shaped ridge which corresponds to the external furrow before mentioned. Overhanging somewhat the basal limb of this Y-shaped ridge posteriorly there is seen a smooth rounded protuberance of more or less pyriform outline. Subsequent examination has shown this to represent the only persisting portions of the thalamus. At its base this thalamic mass becomes flattened from within outward, and is continuous with the thickened basal portion of the cerebrum as seen in the diagram of the brain in sagittal section (fig. 11).

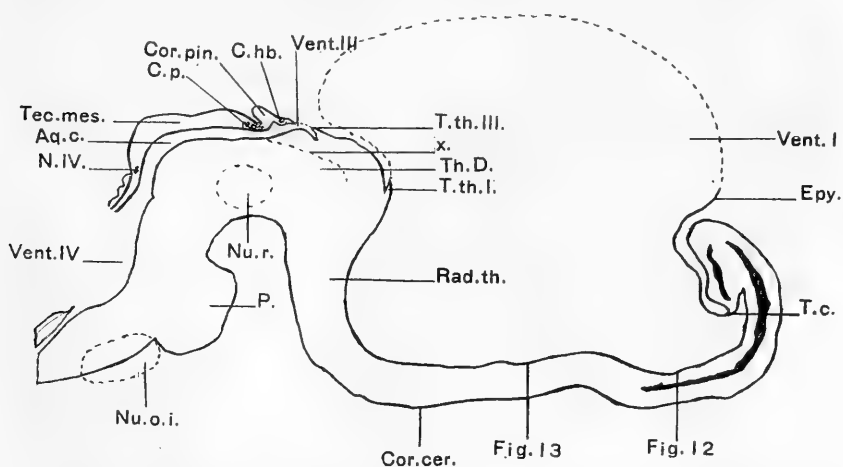
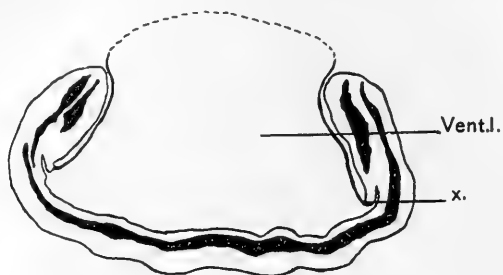
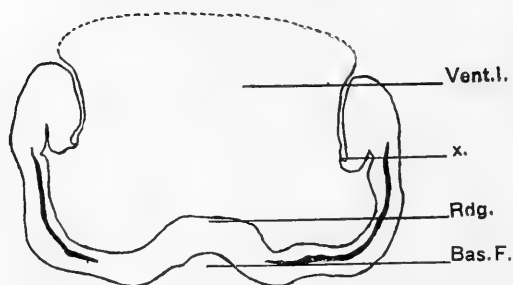


Fig. 11 Diagrammatic medial sagittal section through the entire brain. The levels at which the following coronal sections (figs. 12 and 13) were taken are indicated. *Aq.c.*, iter; *C.hb.*, habenular commissure; *Cor.cer.*, cortex cerebri; *Cor.pin.*, pineal body; *C.p.*, posterior commissure; *N.IV.*, trochlear nerve; *Nu.o.i.*, inferior olive; *Nu.r.*, red nucleus; *P.*, pons; *Rad.th.*, ventral thalamic radiations; *T.c.*, taenia cerebri, or point of attachment of thin cerebral roof (*Epy*) to inner pillar of cerebral margin; *Tec.Mes.*, midbrain roof; *Th.D.*, dorsal thalamic mass; *T.th.I.*, attachment of cerebral roof to thalamus; *T.th.III.*, attachment of thin roof of third ventricle to thalamus; *Vent.I.*, cerebral ventricle; *Vent.III.*, third ventricle; *Vent.IV.*, fourth ventricle; *X.*, endymal diverticulum from iter. $\times \frac{2}{3}$.

The roof, which is made up of ependyma, pia and fibrous tissue fused together, is everywhere quite thin. Its relations to the thickened base are best brought out in the diagrams (figs. 11, 12 and 13). These represent respectively a sagittal and two transverse sections of the brain.



12



13

Fig. 12 Coronal section through fore-part of cerebral vesicle. *Vent.I.*, cavity of cerebral vesicle; *X.*, marks point of attachment of thin roof to inner pillar of thickened recurved cerebral margin. $\times \frac{2}{3}$.

Fig. 13 Coronal section through mid-part of cerebral vesicle. *Rdg.*, ridge in ventricular cavity corresponding to external furrow (*Bas.F.*). The relations of this ridge and furrow are further illustrated in figures 50 and 51. Other letters as in figure 12. $\times \frac{2}{3}$.

It will be seen in these diagrams that the thickened basal portion of the cerebral vesicle presents an arched margin, and the roof is not attached to the apex of this arch but to the base of the inner pillar. This recurved margin subsequently has been identified as a modified hippocampal formation. Thus the point of attachment of the thin roof will represent the fimbria.

The relation of this roof to the thalamic mass as subsequently determined may be seen by comparing a sagittal section (fig. 11) with a surface view from above and in front (fig. 14).

It will be seen that there is a discontinuity of the ventricular system in this region so that the large cavity of the primary fore brain vesicle is not in connection with the iter. The line of attachment of the thin roof to the surface of the thalamic mass

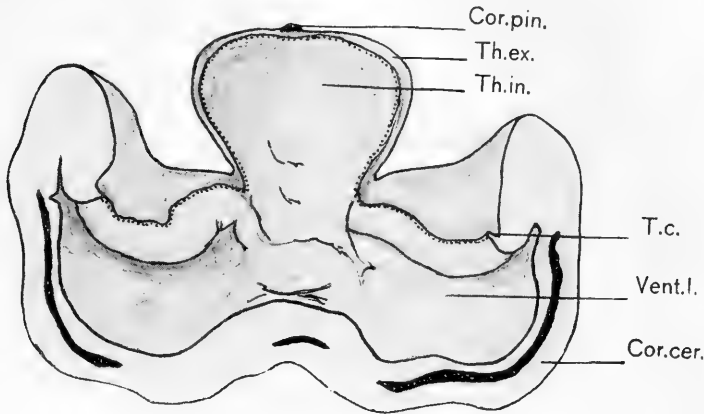


Fig. 14 Diagram of posterior portion of forebrain seen from above and in front. The thin roof has been removed and the point of its attachment to the cerebrum and thalamic mass is indicated in dotted lines. *Cor.cer.*, cortex cerebri; *Cor.pin.*, pinal body; *T.c.*, taenia cerebri; *Th.ex.*, extraventricular portion of the thalamus; *Th.in.*, intraventricular portion of the thalamus; *Vent.I.*, cavity of cerebral vesicle.

corresponds to the taenia thalami. The taeniae become continuous with one another some distance in front of the pinal body. At the point of junction between the thalamic mass and the cerebrum, the roof is attached to the margin of the latter structure and the taenia thalami becomes continuous with the fimbria.

In the diagrams (figs. 11, 12 and 13), the basal plate of the cerebrum is represented as split into two laminae. This splitting apparently occurred during fixation and the line of cleavage passes in most cases through the portion representing the medullary center.

It is to be noted that the posterior poles of the so-called basal lobes of the cerebral vesicle project backward for a short distance on either side of the thalamic mass and peduncular region.

In connection with the foregoing description it must be borne in mind that beside the malformations which are the direct outcome of the cyclopic condition, others are present which are consequent on the internal hydrocephalus. It is to this factor that we must look for the cause of the marked dilatation of the forebrain vesicle with the bulging of its thin walled roof, and probably also for the lack of continuity in the ventricular system. These points will be more fully dealt with later.

Brain stem and cerebellum

The midbrain, as may be seen in figures 48 and 49, is plainly visible from above and on account of its thick pial covering shows no external indications of division into corpora quadrigemina. The marked flexure which obtains between the primary forebrain vesicle and the brain stem causes the peduncular region to be completely hidden in a ventral view of the brain. No cerebral peduncles are present.

Both vermis and lateral lobes of the cerebellum are well developed. Their various subdivisions show but little departure from the normal. The pons, which cannot be distinctly seen in the figures on account of the marked flexure of the brain stem, is also well developed.

The absence of the pyramids and the great prominence of the olives are the only external features in which the medulla appears to vary from the normal. The attachments of the cranial nerves could not be made out in the gross as they had been removed, together with the vessels and pia, before the material came into my hands but subsequent to the drawings shown in figures 48 to 51.

It is to be noted that the marked abnormal flexure of the brain stem in the region of the midbrain is apparently due to the non-development of the cerebral peduncles.

TECHNIQUE

Without further examination, the brain stem and thalamic mass were imbedded in celloidin. When this had been hardened sufficiently it was subdivided into blocks of convenient size and serial sections made 45 microns in thickness. Sections were selected at suitable intervals (about every tenth, but in places every fifth section) and stained by a modified Weigert method and counterstained with Upson's carmine. For comparison, a similar series was prepared from the brain stem and left cerebral hemisphere of a normal full term foetus.

Blocks also were cut from selected areas of the cortex of the cerebral vesicle and sections of various thicknesses were examined. For comparison, sections were studied from the cortex of the right cerebral hemisphere of the normal foetus mentioned above. In each case the following stains were used: modified Weigert, modified Nissl, and a simple hematoxylin-eosin stain.

The drawings of the microscopic preparations from the brain stem and cerebral cortex have been made with the aid of a Leitz projectoscope.

MICROSCOPIC DESCRIPTION OF THE BRAIN

Brain stem and cerebellum (figs. 24 to 47)

On comparison with sections taken from the normal brain stem at birth, this series shows several outstanding points of difference. There is a complete absence of the crura cerebri in the midbrain, the fasciculi longitudinales pyramidales in the pons and the pyramids in the medulla. The marked S-shaped flexure of the brain stem causes sections, which are transverse to the long axes of the medulla and midbrain, to be oblique and horizontal in the intervening regions. There is also a marked deficiency in the number of well medullated fibers present throughout. The development of the myelin sheaths has evidently been retarded. In the region of the midbrain the pia has been greatly thickened and forms a thick capsule which entirely masked the surface markings over this area in the recent state.

Cranial nerves. As has already been mentioned in the gross description, the olfactory bulbs and tracts are entirely wanting.

The optic nerve is represented by a few fibrous strands which pass from the bulbus oculi at the region of entrance of the central artery into the surrounding connective tissue of the orbit. As no microscopic examination was made of these, it is impossible to say whether any true nerve fibers were present. No connection existed between the eye and the central nervous system.

The deep connections of the cranial nerves from the oculomotor caudad are practically normal and will be but briefly noted here.

The oculomotor nerve (*N.III*, figs. 36 to 39) is essentially normal in its origin. Cephalad, the oculomotor nuclei (*Nu.N.III*) are in relation with a well marked nucleus of the posterior longitudinal bundle or nucleus of Darkschewitsch (*Nu.f.l.m.*). Some of the more caudal fibers of this nerve have a crossed relation. The emergent fascicles do not pierce the red nucleus but curve around its caudal and mesial surfaces.

The trochlear nerve (*N.IV*, figs. 32 to 35), although very small, can be made out readily. Its nucleus (*Nu.N.IV*) is in relation to the posterior longitudinal bundle and its root fibers pass towards the superior medullary velum on the median aspect of the mesencephalic root of the trigeminal nerve. The decussation in the velum is quite normal.

The trigeminal nerve (*N.V*) is normal and its relations are well brought out in figures 24 to 37.

The abducent nerve (*N.VI*, figs. 32 to 36) shows no departure from the normal. The connection between its nucleus (*Nu.N.VI*) and the superior olive (*Nu.o.s.*) is also to be made out.

The facial nerve (*N.VII*, figs. 32 to 39) is normal in all its relations. The emergent fascicles on the right side are arranged around a small artery.

The vestibular nerve (*N.VIII, vest.*, figs. 32 to 39) is normal in its relations and connections. The cochlear nerve (*N.VIII, coch*) is also normal and the dorsal and ventral cochlear nuclei are quite evident (*Nu.coch.d.* and *Nu.N.coch.*). The corpus trapezoideum (*c.t.*), superior olive (*Nu.o.s.*) and lateral lemniscus (*L.l.*) are well shown, and the continuity of the superior olive with the

nucleus of the lateral lemniscus (*Nu.l.l.*) is also apparent. The striae acusticae (*s.a.*) are but poorly medullated.

The glossopharyngeus and vagus nerves (*N.IX* and *X*) together with the tractus solitarius (*T.s.*), nucleus alae cinereae (*Nu.a.c.*) and nucleus ambiguus (*Nu.amb.*), are shown in figures 27 to 32.

The accessory nerve (*N.XI*) shows a somewhat anomalous condition of development. But few fibers of its medullary portion could be distinguished on the left side. On the right, these fibers are grouped peripherally in a compact bundle which is well shown in figure 24, lying just within the ventral border of the substantia gelatinosa (*s.g.*). These fibers apparently take their origin from the central gray matter lateral to the central canal. No definite nucleus could be distinguished in this region but these fibers are clearly not related to the nucleus ambiguus; vide Cajal (3).

The hypoglossal nerve (*N.XII*) and its nucleus (*Nu.N.XII*) are normal in their relations. There is no sharp line of demarcation between the hypoglossal nucleus and the motor nuclei of the anterior horn in the upper cervical cord. The accessory nucleus of Roller is also to be made out.

Arcuate nuclei (*Nu.a.*, figs. 25 to 34). These bodies are prominent in the lower medulla and are here somewhat larger than normal. On the appearance of the inferior olive, however, they diminish in size and at the level of the pons become continuous through the nucleus of the raphe with the nuclei pontis.

Inferior olive (*Nu.o.i.*, figs. 27 to 35). These form very prominent projections on the ventral aspect of the medulla. They are somewhat larger than normal. As the series in this region was not absolutely complete, the exact antero-posterior diameter could not be determined but it would not be much less than 8 mm. The greatest dorso-ventral diameter is 5.6 mm and the transverse diameter is 3.8 mm. These dimensions in Sabin's model (20) were 7.5 mm., 4.48 mm., and 6.5 mm., respectively. There has been, therefore, an increase of the dorso-ventral at the expense of the transverse diameter. This change of form is probably due to the absence of the pyramids. In transverse

section it is seen that the olives differ from the normal in showing three very definite outpouchings—a dorsal, a ventral and a lateral. The fibers arising from the olive are quite lacking in myelin. The mesial and dorsal accessory olives are present in practically normal relations.

Lemniscus system. The decussatio lemniscorum (*Dec.l.*), the stratum interolivare lemnisci (*S.i.l.*) and the mesial lemniscus (*L.m.*) up to the level of the anterior portion of the red nucleus (*Nu.r.*) are essentially normal. At about the caudal end of the nucleus ruber, a well marked strand of fibers is given off from the lateral part of the main sheet of the fillet. This has been identified as the superior lemniscus (*L.s.*, fig. 39). It passes up to end in the region of the superior colliculus. Beyond the nucleus ruber the lemniscus cannot be traced as a definite fiber system. Here its fibers become scattered and are lost in the dorso-lateral portions of the thalamic mass.

Fasciculus longitudinalis medialis (F.l.m.). This fiber system is prominent throughout the series caudad to the posterior commissure. It is the most completely medullated tract in the brain stem and on this account can be easily separated from the lemniscus in the stratum interolivare. Its relations throughout are normal and it is traceable, together with fibers from the stratum album profundum of the midbrain, into the posterior commissure. Above the oculomotor nucleus (*Nu.N.III*) it is related to a well developed nucleus (*Ny.f.l.m.*, figs. 38 to 40) having the same relations as the nucleus of the posterior longitudinal bundle in normal sections (Darkschewitsch).

Corpus trapezoideum (C.t., figs. 35 to 37). The trapezoid body and the lateral lemniscus (*L.l.* figs. 33 to 37) together with their associated nuclei are quite normal in their relations and course. The lateral lemniscus terminates in the well marked nucleus of the inferior colliculus (*Nu.c.i.*, figs. 33 to 36). No brachium of the inferior colliculus is present.

Cerebellum and peduncles. The cerebellum shows a well developed dentate nucleus (*Nu.d.*) together with globose (*Nu.g.*, figs. 31 to 32) emboliform (*Nu.emb.*, figs. 32 to 34) and roof nuclei. There are almost no medullated fibers to be found in the

cerebellum other than those entering by way of the corpus restiforme (*Cr.*, figs. 27 to 37) and those arising in the dentate nucleus. Even the medullary center of the flocculus is lacking in myelin, whereas in the normal term foetus this area is usually well medullated.

The relations of the cerebellar peduncles are essentially normal. The corpus restiforme is but slightly medullated and shows a very distinct and circumscribed nucleus (*Nu.cr.*, figs. 34 to 35) after its entrance into the cerebellum. The pons, which is well developed, contains no medullated fibers. The brachia conjunctiva (*Br.c.*, figs. 32 to 37) are readily distinguished, arising in the nucleus dentatus and coursing ventrad and cephalad to decussate caudad to the red nucleus. Their fibers are poorly medullated.

Nucleus ruber (*Nu.r.*, figs. 37 to 42). The red nucleus is well formed and prominent in the midbrain region. Owing to the absence of the crusta and also of a well marked substantia nigra, this nucleus is only separated from the periphery by a very short distance. It is to be noted that the emergent fibers of the oculomotor nerve do not at any point pierce the substance of the red nucleus as they do in the majority of cases normally. The cephalic end of this nucleus is in relation with the lateral nucleus of the thalamic mass.

Superior colliculi (*C.s.*, figs. 35 to 39). These form prominent projections which are completely covered over by a thickened layer of pia (*P.*, figs. 31 to 46) which surrounds the midbrain region. The cellular elements are but poorly differentiated. A few medullated commissural fibers pass across the mid-dorsal line above the stratum album profundum (*s.a.p.*). In the mid-line dorsal to the aqueductus (*Aq.c.*) in this region there is a well marked oval mass of embryonal cells (*Ng.*, figs. 36 to 39).

The dorsal tegmental decussation of Meynert (*D.t.d.M.*, fig. 37) is well shown ventral to the oculomotor nuclei. The ventral tegmental decussation was also distinguished but is not shown in the figures.

Fasciculus retroflexus of Meynert (*F.r.M.*, figs. 38 to 46). This bundle forms a prominent landmark in the region of junc-

tion between the midbrain and diencephalon. The corpora habenulae are not well developed, but on each side their site is marked by the beginning of the fasciculus retroflexus. The bundle passes down and comes into relation with the dorso-mesial surface of the red nucleus. From this point onwards it is applied to the mesial surface of this nucleus in its course to the ganglion interpedunculare. At no point does the fasciculus pierce the red nucleus. Throughout the major part of its course this bundle is accompanied by a small collection of gray matter, as is usually the case, normally. The ganglion interpedunculare consists of a somewhat diffuse collection of cells between and ventral to the red nuclei toward their caudal ends.

Thalamic mass

Cephalad to the red nucleus there is found a large irregularly arranged nuclear mass, in the lateral portions of which the lemniscus medialis becomes lost. This area may be roughly divided into a dorsal cellular portion and a ventral fibrillar area.

The dorsal cellular area. Posteriorly, the habenular bodies (*Nu.hb.*, figs. 44 to 46) may be distinguished, together with the fibers of the fasciculus retroflexus of Meynert which arise in these nuclei.

The cells making up the thalamic nuclei are of two varieties:

a. Both large and medium sized, well developed multipolar cells whose cytoplasm takes the carmine stain deeply and which do not differ in any marked degree from those found in the pulvinar of the normal thalamus.

b. Scattered between these large cells are numerous small embryonic or neuroglial elements, having a small amount of cytoplasm which does not take the carmine stain deeply. These cells are far more numerous than the large multipolar variety, and are found both in the dorsal nuclear portion of the thalamus and in the ventral field, though far more abundant in the former area. The large cells, on the contrary, are almost entirely confined to the dorsal nuclear area.

These cellular elements are arranged in irregular groups so that it is possible to distinguish certain irregularly arranged nuclei in the dorsal area.

In the anterior portion, the dorsal area is divided into two large irregular lateral nuclei (*Nu.lat.Th.*, figs. 41 to 46) and a smaller mesial nucleus (*Nu.med.Th.*).

Caudally the lateral nuclei are further subdivided so that it is possible to distinguish three nuclei in this region. These may be termed for descriptive purposes, dorsal (*Nu.lat.1*), lateral (*Nu.lat.2*), and central (*Nu.lat.3*) nuclei of the lateral mass. The mesial nucleus (*Nu.Med.Th.*) is present in the caudal portion in essentially similar relations.

It is to be noted that these thalamic nuclei do not come into relation with the cortex cerebri at the junction of the thalamic mass and the cerebral vesicle, but are separated from it by the ventral thalamic radiation.

The ventral fibrillar area. This area occupies but a small space in sections through the more caudal part of this region, but increases in size as one passes forward by the addition of fibers arising in the dorsally placed nuclei. It is made up of a complex of both medullated and nonmedullated nerve fibers, which, in the caudal portion, are twisted into irregular whorls and tangles. It is remarkable that, in this region, irregular strands of poorly medullated fibers may be seen in numerous places piercing the outer limiting layer of neuroglia in the ventral region and ramifying within the thickened pia (β , figs. 42 to 44).

Passing forward, the thalamic mass becomes united to the cerebral vesicle by a narrow peduncle. This peduncle is seen to be made up almost entirely of ventrally coursing fibers continuous posteriorly with those of the ventral area of the thalamic mass. These fibers must be taken to represent an atypically developed thalamic radiation (*Rad.Th.*, figs. 41 to 47).

The major portion of the fibers of the thalamic radiation are applied to the ventral surface of the cerebral vesicle on which they rapidly spread out and come to an end. The ventral surface of the cerebral vesicle represents the free surface of the cortex. Thus most of the fibers arising in the thalamic mass (*Rad.Th.*) pass directly into the zonal or plexiform layer of the cerebral cortex (*St.z.*, figs. 46 to 47).

Cerebral vesicle

There is no appearance whatever of corpus striatum, rhinencephalon, or in fact of any of the structures developed ventral to the recessus neuroporicus in this region under normal circumstances. The term 'rhinencephalon' is here used to indicate the basal structures of the forebrain in most intimate connection with the olfactory nerve and does not include pallial olfactory centers. It is only for the sake of convenience in the present description that I consider myself justified in employing the term rhinencephalon in this fashion; for when used in the above sense it effectually defines the limits of those areas which are quite absent from this brain.

Cortex cerebri. The basal portion of the cerebral vesicle varies in thickness from about 10 mm. at its thickest at the point of entrance of the thalamic radiations, to about 5 mm. at its thinnest. The cortex shows well developed cell lamination, and the types of lamination vary in different regions. It is impossible, however, to identify these areas as representing any of the histologically differentiated regions to be found in the normal cortex at birth.

There is practically no medullated tissue to be found throughout the cortex. Indeed, the only area in which it is prominent at all is at the point of entrance of the thalamic radiations. In this region in figures 46 and 47, the cortex of the left posterior pole is shown cut tangentially. In the second layer of the cortex here, numerous groups of cells (*C.isl.*) disposed in irregular, more or less circumscribed areas, are to be noted. These islands are made up of two kinds of cells; (a) very numerous, small, embryonic elements, and (b) large polymorphic multipolar cells. In figure 15 this arrangement is brought out in transverse section. Only over this area of the cortex in this specimen were these large polymorphic elements to be found predominating in the stratum which is normally the layer of small pyramids. It would thus appear that the presence of the fibers of the atypical thalamic radiation has exerted an influence upon the growth of these neurones. Just what connection, if any, exists between these cells and the thalamic fibers could not be ascertained. The tis-

sue was not in a favorable condition for metallic impregnation and, although numerous attempts were made, no successful preparations were obtained.

Everywhere in this case the cortex is markedly thickened. The thickest cortex at birth (at least in the case I have used as control) is over the central area. Here the cortex is about 2 mm. in thickness, while the thinnest cortex in the cyclopiian foetus measures 3 mm. or more, the line of demarcation between cortex and medulla being very indefinite. The thickening is mostly the result of an increase in the deep polymorphic layer of cells, although all layers show an increased thickness.

When examined at a low magnification the wall of the cerebrum is everywhere seen to be divisible roughly into five strata. This lamination is illustrated somewhat diagrammatically in figure 18. The strata appear as follows: (1) an outer layer comparatively free from cells and varying in thickness in different areas, (2) a stratum rich in cells but whose elements tend to become arranged in large irregular groups (3) a layer of densely packed cells, (4) a layer whose cellular elements resemble somewhat in arrangement those of the second stratum, and (5) a layer representing the medullary center.

For a more detailed description, sections have been selected from two histologically distinct areas of the cortex (figs. 15 and 16). Figure 17 shows a section through the precentral area in a normal full term foetus for comparison as to thickness and general arrangement of elements. These drawings were made with the aid of a Leitz projectoscope and are each at a magnification of 65 diameters.

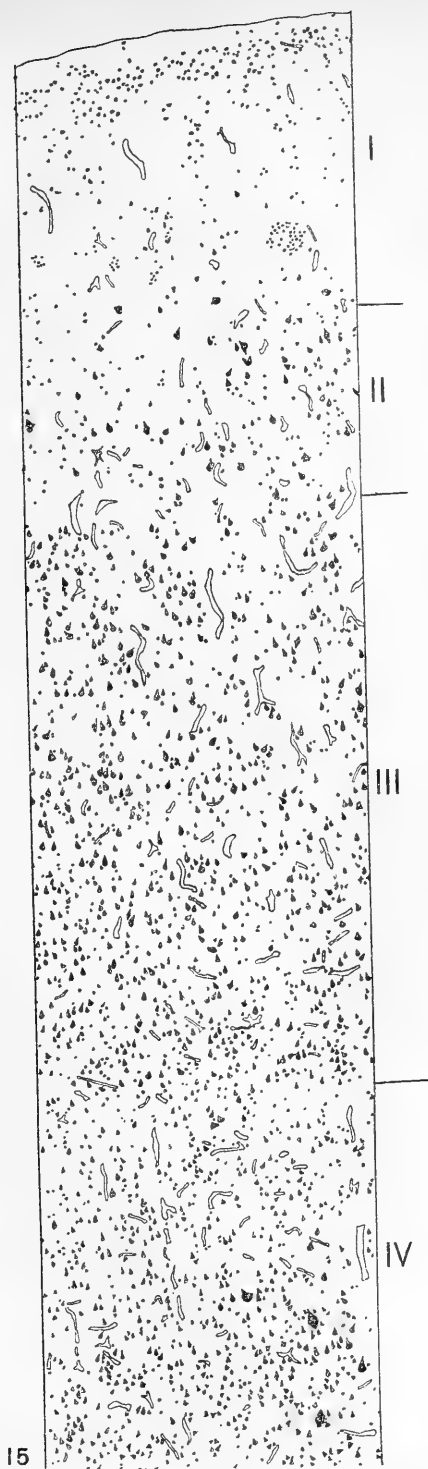
Figure 15 has already been referred to and is a section taken through the cortex near the junction of the thalamus and cerebral vesicle. The plexiform layer (I) at its periphery shows an irregular layer of embryonic, or more probably neuroglia, elements. Into this plexiform layer stream thalamic fibers. The boundary between layer (I) and the next stratum (II) is not sharp. In the second stratum (II) occur the groups of large polymorphic elements mentioned above. The third layer (III) is made up of medium and small pyramidal cells and embryonic elements. It is slightly thicker than layers I and II combined

and the average size of its cell elements become progressively smaller from without inwards. The fourth or polymorphic layer (IV) in the figure appears to be further subdivided into a more superficial zone of scattered small cells and a deeper more compact zone. This is not the case, however, and this appearance is due to some of the elements in this layer being arranged in groups of irregular size, the cells in which are more closely packed than in the intervening spaces. Only a portion of one of these groups appears in the drawing and included in it are shown several very large well developed pyramidal cells. These large pyramidal cells occur singly or in groups over this area and are situated at about the level of the middle trisection of the polymorphic layer. The whole depth of this layer is not shown in the figure. The cells referred to exceed in size the largest cells found in the normal foetal cortex used as control.

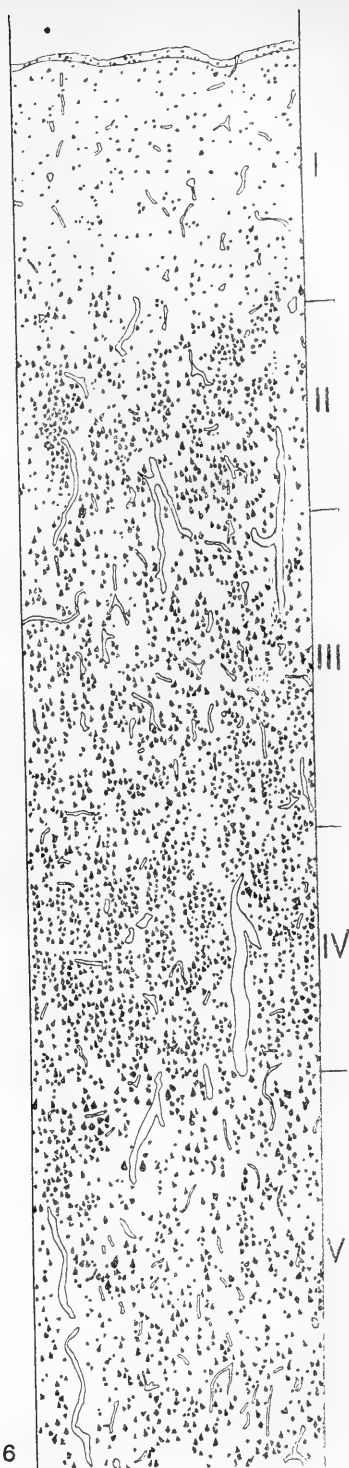
Figure 16 is taken from the so-called anterior lobe of the cerebral vesicle at a point about an inch from the arched margin of the same in the mid-line. The plexiform layer (I) is here quite sharply marked off from the subjacent cell layer. In this layer (II) the elements are somewhat closely packed and arranged in irregular groups. It varies considerably in thickness at different points at the expense of layer III. In the latter layer the arrangement of cells is somewhat looser and it apparently corresponds to Bolton's fourth layer or inner fiber lamina (2). Layer IV is made of closely packed small cells having a quite characteristic embryonic arrangement in the form of irregular rows at right angles to the surface of the cortex. The line of demarcation between this layer and the preceding one is very easily made out and in places is almost as sharp as that between the plexiform layer and layer II. Layer V, the whole thickness of which is not shown in the figure, is the thickest of the cortical laminae, and combined with layer IV makes up more than one-half of the total thickness of the cortex. Its cells are arranged in a

Fig. 15 Cyclopiian cortex cerebri. Section taken from the region of junction of the cerebral vesicle and the thalamus. Explanation in text (page 223). $\times 65$.

Fig. 16 Cyclopiian cortex cerebri. Section taken from the 'anterior lobe.' Explanation in text. $\times 65$.



15



16

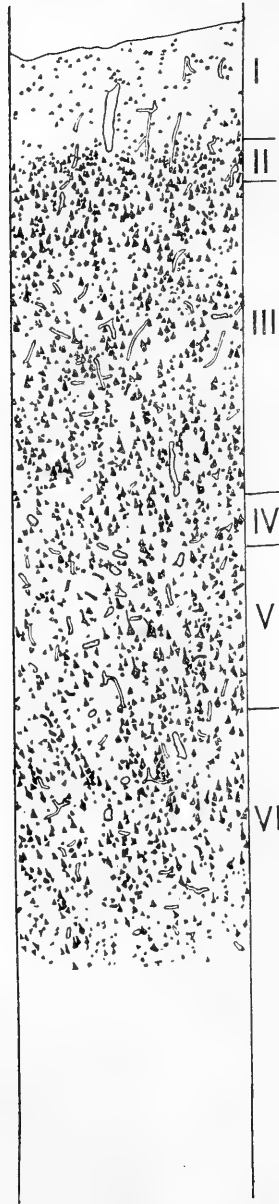


Fig. 17 Cortex cerebri of normal term foetus. Area precentralis B_1 (Elliot Smith). *I.*, plexiform layer; *II.*, small pyramid layer, the cells here are small and many are quite embryonic; *III.*, layer of medium and large pyramids; *IV.*, granule layer which is a very thin and indistinct lamina; *V.*, Betz cell layer; *VI.*, polymorphic layer. $\times 65$.

scattered fashion and occasionally form irregular groups of various sizes. There are no giant cells in this stratum over this area of the cortex.

The blood supply of the cyclopiian cortex, as evidenced by the size and number of blood vessels, seems to be quite as rich as is the case normally.

With regard to the atypical course of the thalamic projection fibers it is to be noted that their presence in the plexiform layer is only the result of the altered relations between the pallium and the thalamus. Normally in mammals the thalamic fibers, to reach the pyramidal dendrites, must traverse the cortex from within outwards. Harrison (10) has shown that nerve processes will develop readily even when the neurone is situated in an entirely strange environment. Under the altered form relations, then, in this case it is but natural that these fibers should still retain their growth energy and pass ventrad along the only course open to them to gain the cerebrum (figs. 46 and 47). On reaching the latter, they mostly take the shortest way available by which they can reach the dendritic processes of the pyramidal elements, namely, by coursing in the zonal layer. The apparent influence of this contact has already been noted.

It is of interest to note here that the passage of both efferent and afferent projection fibers in the plexiform or zonal layer of the cortex is the normal condition obtaining in Amphibia. In these forms the zonal layer represents the only white layer of the cortex visible in transverse section (4). The cortical neurones occupy the space between the zonal layer and the ependyma. Their axones curve outwards to reach the peripheral white matter, while their dendrites come into contact with the afferent projection fibers in this layer also.

In the more caudal portions of the thalamus are found numerous fibers which in view of crowding have been unable to reach the cerebrum. Even under such adverse conditions the growth of these fibers was not arrested. They are here woven together into knots and tangles, and numbers of them have already been described as even piercing the limiting layer of neuroglia and ramifying in the pia (β , figs. 42 to 44).

Owing to the great modification of cell lamination brought about by the presence of thalamic projection fibers in the plexiform layer of the cortex in certain regions, it is not considered advisable to attempt to compare in detail the type of lamination found in such regions with that normally occurring in the developing cortex. In areas remote from such disturbing influences, however, such comparison may, I think, be safely made.

In figure 16 it was noted that layer III apparently corresponded to Bolton's inner fiber lamina (2). The inner fiber lamina in the normal developing cortex is a layer which develops as the result of a separation of the cortical neuroblasts into an outer and an inner cell lamina. The polymorphic layer of the normal adult cortex is derived from the inner cell lamina, while practically the whole cortex above the inner line of Baillarger is derived from the laminae superficial to this. If, then, layer III represents the inner fiber lamina, it would appear that the outer layers of the cortex in this case are in a state of sub-evolution. For the total thickness of layers I to III inclusive is less than that of the much thickened polymorphic layer (IV and V). The somewhat closely packed lamina of cells constituting layer IV, which is well developed over most areas of this cortex, appears to be peculiar to this case and not comparable to any layer normally present at term. In the case reported by Naegeli (17) however, a layer of apparently similar nature is shown in his figure 42. It would thus seem that the cortex had begun its development from within outwards in this region, as is normally the case.

At first sight it appears difficult to account for the greatly increased thickness of the cortex in a cerebrum in which cell differentiation has been described as subnormal. This difficulty is only an apparent one for, as will be subsequently pointed out, there is evidence in this brain to show that at least the greater part of the undifferentiated normal pallial anlagen are present. The surface area of the cortex, however, has not been increased by the formation of cerebral convolutions. Thus a large number of cell elements have to accommodate themselves over a limited area, resulting in the increased thickness of the cortex which

otherwise is characterized by subnormal development. As will be subsequently noted in a future communication, there are reasons for regarding this thickening in some areas as being also partly due to hyperplasia.

Under normal circumstances Bolton (2) has pointed out that the cells in the cortex of the term child are less crowded than are those in the cortex of the developing foetus. As an important factor in reducing this aggregation of cells he points to the increased superficial area of the cortex at term due to the maturing convolitional pattern, "and the consequent smaller number of cells in a section of the same thickness."

Bolton and Moyes (3) have shown that the first large well developed cells in the cortex are the Betz cells and that these are prominent as early as the eighteenth week of foetal life. They are situated in the basal portion of the inner fiber layer. These authors also express an opinion that the sensory or afferent fibers to the cortex are in all probability developed before the motor or efferent fibers from the cortex.

In this case it has been shown that in those areas reached by the thalamic fibers, there is a marked tendency toward atypical overgrowth in certain neurones of the superficial layers with which these fibers come in contact. The presence of afferent fibers thus influences the growth of cortical neurones. Ordinarily these afferent fibers must enter through the basal portion of the cortex. It is in this basal portion of the cortex that the first well differentiated neurones appear and it is the basal laminae of the cortex that are the first to be evolved. It would be interesting to determine how far the tardy ontogenetic development obtaining over some areas of the normal cortex were dependent upon the late appearance in these areas of afferent projection fibers.

Naegeli (17) has described numerous well developed pyramidal cells in the cortex in his case, which have attained a considerable degree of differentiation and are possessed of short axone processes but which do not come into relation with any projection fibers from the thalamus, none of which, he says, gained the cerebrum. He has termed this growth process 'self-differentia-

tion,' as opposed to 'dependent differentiation,' using the terms suggested, as he says, by Roux (19).

In this case, in some of the more basal portions of the cortex which are lacking in medullated fibers of thalamic origin, there are found in the deeper strata numerous giant pyramidal elements. These cells are much larger than any similar elements to be found in the normal new-born cortex, and each possesses a large vesicular nucleus with well developed karyosomes, while in the cytoplasm Nissl bodies are well formed and prominent. Although no medullated fibers of thalamic origin could be made out in such sections, it is very doubtful if these large elements can be considered as cells showing only 'self-differentiation;' they are probably influenced by non-medullated afferent fibers. On the other hand, in areas quite removed from possible thalamic influence as for example, the anterior portion of the rim of the cerebral cup, the smaller, more numerous and generally under-developed elements, making up the thickness of the cortex may, I think, be classed as 'self-differentiated,' in the sense of Roux.

The giant cells referred to in the preceding paragraph, which are located in the deep cell lamina of the cortex, are strikingly like the large efferent perikaryons described as Betz cells in the normal cortex of mammals. It has been shown by Bolton (3) that in the evolution of the cortex, those laminae situated above the level of the Betz cells, are the site of greatest differentiation and growth in the higher mammals and man. It thus happens that the increased cortical thickness causes these large efferent cells to lie more deeply in the human cortex than in that of lower forms. If then the giant cells in this cyclopic cortex represent the cell bodies of potential efferent neurones of the Betz type, the location at such a cortical level would be a further argument against any theory of phylogenetic reversion. An accurate knowledge of the number, arrangement and distribution of these cells would aid in determining how far such a comparison as the above is justified. In the event of the presence of sufficiently differentiated cortical tissue in any of the other cases of cyclopia at present in my possession, a complete topographical survey of such will be made. It would also be of interest to note what

relation exists between the development of the corpus striatum when present, and such efferent neurones as those under discussion.

One other point may here be noted in connection with the examination of this cortex. It has been found that the character of the lamination, the thickness of the cortex and the condition of cell development, vary in different regions. Two of these histologically distinct areas are illustrated in the figures. It would seem that these areas are not sharply marked off from each other but are separated by transitional zones. However, as no complete topographical survey was made, this point was not definitely determined. Thus, histologically distinct areas are present but, with the exception of the dentate and fimbriodentate fissures, there is a complete absence of true sulci. This, then, is further evidence, it would seem, of the truth which Bolton has pointed out, namely, that the development of the convolutional pattern is secondary to the differentiation of the cortex into histologically distinct areas.

Cerebral limbi. At the base of the inner pillar of the thickened recurved margin of the cerebral vesicle, the thin roof becomes attached to the edge of the cortex. The relations of the roof in this region have already been noted in the gross description (figs. 11 to 13). Sections were made at intervals along this margin and the relations of the cerebral limbi studied. Drawings of three of these sections are here reproduced. Figure 18 is a section taken in the mid-sagittal plane; figure 19 is a section taken at the junction of the anterior and middle thirds, and figure 20 a section at the junction of the middle and posterior thirds of the left cerebral margin. The outlines of these drawings were made with the aid of a Leitz projectoscope and at the same time lines were drawn marking off the various cell laminae of the cortex. The spaces between these lines were subsequently shaded in free hand, so the details of cell arrangement are only indicated in a somewhat diagrammatic fashion. However, the relative thickness of the cortex and the arrangement of its various strata, as seen under low magnification, are fairly accurately shown.

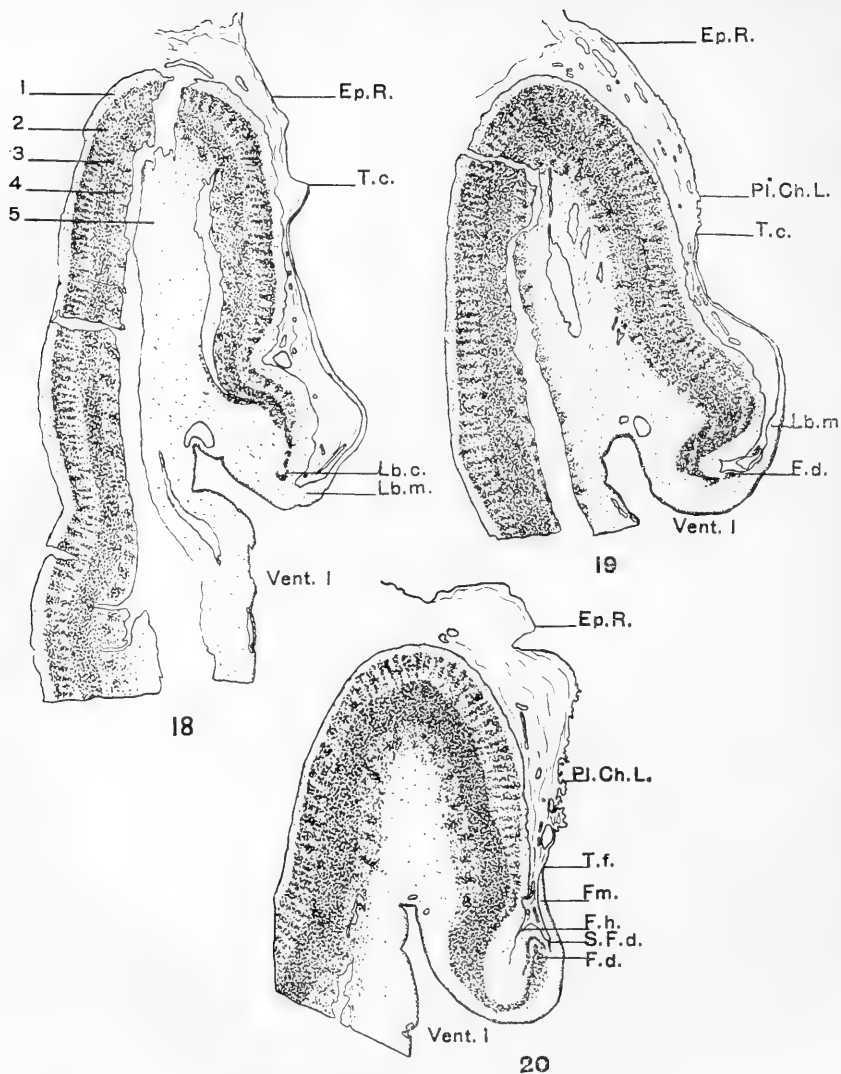


Fig. 18 Section through cerebral margin in mid-sagittal plane. *Ep.R.*, ependymal roof; *Lb.c.*, limbus corticalis; *Lb.m.*, limbus medularis; *T.c.*, taenia cerebri; *Vent.I.*, cavity of cerebral vesicle. Nos. 1 to 5, cortical laminae, explanation in text (page 231). $\times 3.5$.

Fig. 19 Section through cerebral margin in plane of junction of anterior and middle thirds of lateral rim. *F.d.*, fascia dentata; *Pl.Ch.L.*, choroid plexus. Other letters as in figure 18. $\times 3.5$.

Fig. 20 Section through cerebral margin in plane junction of middle and posterior thirds of lateral rim. *F.d.*, fascia dentata; *F.h.*, hippocampal fissure; *Fm.*, fimbria; *S.F.d.*, fimbrio-dentate sulcus; *T.f.*, taenia fimbriae. Other letters as in figure 18. $\times 3.5$.

The major portion of the posterior two-thirds of the cerebral rim on both sides has been found to represent a but slightly modified hippocampal formation (fig. 20). The line of attachment of the thin roof (*Ep.R.*) corresponds to the edge of the limbus medullaris or fimbria (*Fm.*). A well marked fascia dentata (*F.d.*) is present, bounded laterally by the fissura hippocampi (*F.h.*), and mesially by the fimbrio-dentate sulcus (*S.F.d.*). Above the fimbria, the thin roof is invaginated by vascular tissue to form a band of choroid plexus (*Pl.Ch.L.*). This 'choriogenous zone' extends forward only as far as the extreme anterior tip of the fascia dentata (*F.d.*, fig. 19). It is to be noted that if the normal lateral plexus could be straightened out it would represent an area such as is found here and bearing precisely similar relations to the fimbria.

Throughout the major portion of the anterior third of the cerebral rim, the fascia dentata can not be made out. Here the limbus corticalis has not become rolled upon itself (*Lb.C.*, fig. 18). The limbus medullaris (*Lb.M.*) becomes on this account more extensive. No choroid invaginations are present over this area.

The identity of the limbus corticalis between the anterior extremities of the two definitely identified hippocampal areas is as yet obscure. It is hoped that further study in comparison with the other cases in my hands may clear up this point.

As has already been mentioned, no medullated fibers are present in this area of the cortex. In this connection it is to be noted that there was a complete absence of medullated tissue throughout the hippocampal area in the normal term foetus used as control.

Ventricular anomalies

The cavity of the fourth ventricle (*Vent. IV*) is essentially normal in its relations. It is lined throughout by a well developed layer of ependyma.

Turning now to the aqueductus (*Aq.C.*, figs. 34 to 42), it is to be noted that numerous irregular ependymal diverticula arise from its ventral and lateral walls in the region just caudal to the posterior commissure. One of these evaginations passes

ventrad and cephalad for a considerable distance and may be traced into the mesial thalamic nucleus (X, figs. 42 to 47).

Just cephalad to the posterior commissure the pineal recess may be distinguished, and in front of this a few fine medullated fibers cross in the thin roof and constitute the habenular commissure (*C.hb.*, fig. 44).

Immediately cephalad to the habenular commissure, the taenia thalami (*T.th.III*, fig. 45) is to be seen and two small areas of choroid plexus (*Pl.Ch.III*, figs. 44 to 45) project into the small ventricular cavity (*Vent. III*, figs. 44 to 46). Separating these two plexuses is a slightly thickened area in the roof representing the attachment of the anterior limb (dorsal) of the pineal stalk.

In front of this the thin roof of the ventricular cavity has been torn away for a short distance and its relations cannot be accurately followed. However, the roof appears again in the sections somewhat more cephalad and is now seen to be thickened and lacking in choroidal invaginations. Traced forward from this region, the ventricle is seen to end blindly in the dorsal part of the thalamic mass.

It was noted in the gross description that the thalamic mass projected into the cavity of the primary forebrain vesicle and was covered with ependyma. From a study of the sections in this region it now appears that the line of attachment of this ependyma, or rather the line of its reflexion from the surface of the thalamus, approximately coincides with the line of demarcation between the dorsal nuclear mass and the ventral fibrillar area. Thus the greater part of the thalamic mass is extraventricular and is covered by a thick layer of pia and fibrous tissue. The taenia thalami (*T.th.III*), noted just in front of the pineal region, is not continuous with the taenia (*T.th. I*, figs. 45 to 47) over the anterior part of the thalamus to which the thin roof of the forebrain vesicle is attached. These relations are best seen in the diagram of a mesial sagittal section of the brain (fig. 11).

It is thus seen that there is a discontinuity in the ventricular system of the brain and that this interruption occurs in the region

of the third ventricle (figs. 11 and 23). This obliteration of a part of the ventricular cavity is not necessarily the result of the growth conditions producing cyclopia; but probably these growth conditions rendered such an obliteration more liable to occur in this region than elsewhere.

Mechanical considerations

Stockard has shown that the condition of cyclopia may be produced at will in fish embryos in a high percentage of cases by treating the eggs with $MgCl_2$ or $Mg(NO_3)_2$ solutions (24 and 26). He was thus able to study histologically a great number of cases in fish embryos otherwise perfectly normal. His observations showed that "the cyclopic defect is present from the first in the same condition that it will continue throughout development" (25). This statement was subsequently (27) somewhat modified by further experimental work, for it was found that cyclopia could be produced in a small percentage of cases by the action of magnesium chloride, even after segmentation had gone as far as the periblast stage (15 hours). Eggs older than the fifteen-hour stage were not affected by the Mg solutions. Thus, although cyclopia is not necessarily of germinal origin, the growth inhibition begins at so early a stage of embryonic life that it practically amounts to absence of certain areas during development. In other words, cyclopia is not the result of fusion of parts originally separate, but is due to absence during development of certain parts normally separating the eye anlagen.

Cyclopic monsters belong to that class of bilaterally symmetrical beings which have been termed by Wilder (28) 'cosmobia.' Development in these cases proceeds in a orderly fashion until some mechanical difficulty arises which cannot be overcome by the individual and death results. In fish the development in cyclopic forms goes on until the yolk supply is entirely used up, when the animal dies of starvation unless suitable food is artificially provided. In this connection it is also of interest to note that the absence of tissue anlagen does not postulate a non-functional nervous system, for individuals which have been kept alive and observed, reacted to stimuli in a quite normal fashion.

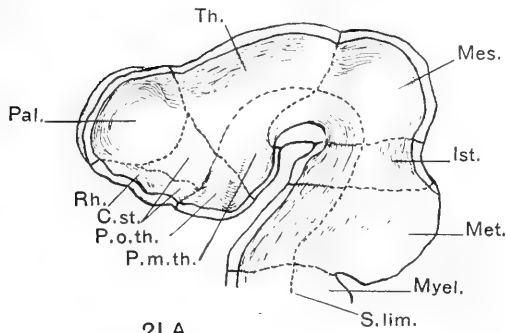
In cycloplan mammals no mechanical difficulties incompatible with life are met until after birth. The animal then usually dies in a short time from interference with feeding and respiratory functions.

Thus, bearing in mind that the cosmobion is governed in all its growth changes by quite definite mechanical laws, an attempt may be made to interpret the form relations in this case. Certain areas have been practically absent during development. If one removed such areas from a model of a very young normal brain and approximated the cut edges, would the resulting malformation be similar to the case in hand?

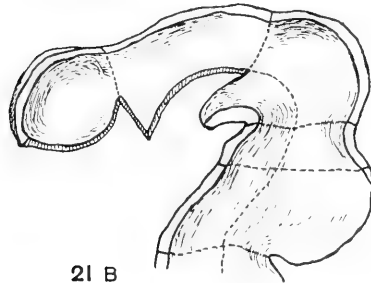
If one examine one of His' models of the brain at the end of the fourth week, it will be seen that the telencephalon is represented by an expanded, thin walled unpaired vesicle, for at this stage the median furrow between the two pallial expansions is not developed. Looking at a mesial sagittal section through such a model, it is possible to mark out on the ventricular surface, with a considerable degree of accuracy, the areas which will later be developed into the corpus striatum and rhinencephalon, the pars optica and the pars mammillaris hypothalami, and the pallium (fig. 21).

It has been shown that in the present case, the pallium alone of these parts is present. Thus, if one cuts out from both sides of a clay model of the brain at the end of the fourth week, these areas which are not developed, and places the two halves remaining in apposition, then by simply pressing the cut surfaces of each half together one has reproduced in all its essentials the form relations obtaining in this cycloplan brain (fig. 22). It must be borne in mind, however, that this is reversing the true sequence of events.

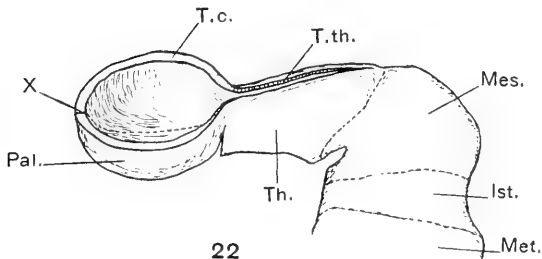
We are left with a cup whose thickened walls are formed by all the pallium, and whose rim is formed by the two original mesial edges, to which of course is attached the much stretched thin roof. These mesial edges in the normal pallium for the most part are the areas in which the hippocampal formations are laid down. It is also along these mesial edges that the lateral choroid plexuses are normally invaginated. So in this case, we



21 A



21 B



22

Fig. 21 A Medial sagittal section of a His' model of the brain at the end of the fourth week (modified from Spalteholz). *C.st.*, corpus striatum; *Ist.*, isthmus; *Mes.*, mesencephalon; *Met.*, metencephalon; *Myel.*, myelencephalon; *Pal.*, pallium; *P.m.th.*, pars mamillaris hypothalami; *P.o.th.*, pars optica hypothalami; *Rh.*, rhinencephalon; *S.Lim.*, sulcus limitans; *Th.*, thalamus.

Fig. 21 B Right half of a His' model of the brain at end of fourth week, from which the anlagen of the rhinencephalon, corpus striatum, pars optica and pars mamillaris hypothalami have been removed. The cut edges are lined.

Fig. 22 Model resulting from the approximation of the cut surfaces as indicated in the text. *T.c.*, taenia cerebri; *T.th.*, taenia thalami; *X*, marks the line of union between the original ventro-mesial edges of pallial anlagen. Other letters as in figure 21A.

find the hippocampal formation developed in the edges of the pallial cup posteriorly, and above the fimbria we find choroidal invaginations of the ependyma.

Turning to the thalamus, it will be seen that the absence of the pars optica and pars mammillaris hypothalami would materially reduce both the volume of the thalamus and the size of its ventricular cavity.

The use of such a model is justified only in so far as it helps to demonstrate the mechanical tendencies that would arise in a brain in which these areas are missing from the start. The brain at this stage of development was taken because, while the relations are simple, it is yet possible to outline the areas occupied by the corpus striatum, and so forth, fairly accurately.

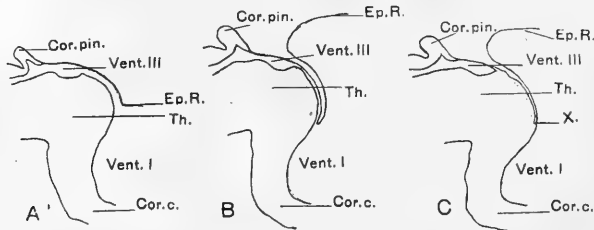


Fig. 23 A B and C. Diagrams of medial sagittal sections through the brain illustrating the hypothetical closure of the connection between the cavity of the cerebral vesicle and the third ventricle. *Cor.c.*, cortex cerebri; *Cor.pin.*, pineal body; *Ep.R.*, ependymal roof; *Th.*, thalamus; *Vent.I.*, cavity of cerebral vesicle; *Vent.III.*, third ventricle; *X*, in figure 23 C. indicates the final point of attachment of the much expanded thin roof to the front of the thalamus.

Sometime during the development of this case the condition of hydrocephalus set in and, as a result, the roof of the cerebral vesicle bulged upward. If one refer now to the diagrams (fig. 23), it can be readily seen how the discontinuity in the cavity of the already reduced third ventricle might be brought about through pressure of the expanding roof of the cerebral vesicle. This thin cerebral roof being confined above by the skull, extended backward as a pocket over the thalamus. Pressure of the fluid contents being transmitted equally in all directions would then tend to close the fore part of the third ventricle. This closure might subsequently be followed by adhesion be-

tween the approximated walls and finally by complete obliteration of the ependyma. Such an explanation is however purely theoretical, as there is no way of determining the time relation between the onset of hydrocephalus and the occlusion of the anterior portion of the third ventricle.

REVIEW OF CASE REPORTED BY O. NAEGELI

In the case reported by Naegeli (17) the cerebrum was represented by an unpaired, thick walled vesicle, having a very slight attachment to the massive thalamus.

The basal ganglia were quite defective and could not be definitely identified. He described, however, a thin plate of embryonic cells in the region of junction between the thalamus and cerebrum which he concludes may represent these structures.

No olfactory bulb or stalk was present but he describes the hippocampal formation as being well developed, and the fimbria as containing medullated fibers. In figure 29 of his report he shows the cornu ammonis in transverse section and it is strikingly similar to sections through this region in the present case. Unfortunately, he has not described the relations of the hippocampus except to mention that it is a distinctly paired formation, so further comparison is impossible. The fornix is also mentioned as an unpaired bundle which divides into symmetrical halves to end in the region of the corpora mammillaria. The relations of this fornix to the fimbriae are not clear.

The cortex of the cerebral vesicle showed a distinctly laminated arrangement of its cells and was quite markedly thicker than normal—the greatest increase in thickness being in the deep or polymorphic stratum. In figure 42 of his report he shows a carmine stained section through the cerebral cortex, in which the cell lamination is almost precisely the same as that described as appearing under low magnifications in the present case. He also found a few poorly medullated radial fibers in the layer of large pyramidal cells. No tangential fibers were present.

The thalamus was massive and of nearly normal form, but the two halves were strongly fused in the mid-line ventrally.

He does not describe any connection between the remains of the third ventricle and the cavity of the cerebral vesicle. From his figures I am led to believe that this connection was interrupted. The attachment of the thalamus to the cerebrum was very slight and wholly basal. Into this region, projection fibers converge from the lateral nuclei of the thalamus, constituting an atypical thalamic radiation. Ventrally the fibers of the two sides decussate and end blindly in the basal region of the cerebrum, which before has been alluded to as the probable representative of the corpus striatum.

It is thus seen that, as in the present case, the third ventricle has been considerably reduced in volume and its connection with the cavity of the cerebral vesicle has also apparently been entirely interrupted.

A single median optic nerve was present which divided posteriorly at a partial decussation into paired optic tracts. Infundibulum, corpora mammillaria and lateral geniculate bodies could be distinguished and a commissure of Meynert is described crossing in the tuber cinereum. Dorsally the habenular bodies were prominent, together with the posterior commissure and fasciculus retroflexus of Meynert. Medullated fibers were found in the taenia thalami (*stria medullaris thalami*).

The thalamic nuclei were not definitely marked out, but the whole thalamic mass was relatively rich in well formed ganglion cells together with more numerous embryonic elements and neuroblasts, being thus similar in these respects to the present case.

From the region of the interpeduncular ganglion caudad, the brain stem was much distorted by a malformation quite independent of the cyclopic condition. A split had occurred in the mid-line, resulting in a more or less complete separation of the brain stem and cerebellum into right and left halves. Relations were further complicated by the presence of an additional flexure in the brain stem and by the bending dorsally of a considerable portion of the split cord so that it came to lie upon the malformed brain stem halves within the skull cavity. A considerable amount of fusion between the cord and underlying brain stem halves was also present.

Notwithstanding these malformations, the cranial nerves from the oculomotor caudad and most of the fiber systems with their nuclei could be identified in the brain stem. There was a complete absence, however, of the pyramidal system throughout.

Naegeli calls attention to von Monakow's (16) classification of fiber systems and nuclei into phylogenetically old and phylogenetically young groups, and points out that only the former are to be identified throughout in his case.

He concludes from the striking resemblance between the arrangement of structures in the forebrain region in his case and those obtaining in the forebrain of teleosts, and also from the complete absence of so-called phylogenetically young fiber systems, that the condition of the brain in cyclopia may represent an arrest of development at a phylogenetically early stage.

It is evident from the above that the prosencephalic disturbance here noted, dependent upon the cyclopic condition, was not so extensive as in the present case.

CONCLUSION

In the case I have reported, there is a very marked superficial resemblance between the forebrain vesicle and the telencephalon in teleosts. The resemblance, however, is only superficial, for the thickened basal structures present in this case are altogether pallial. In teleosts the basal nuclei form the bulk of the thickened base of the telencephalon, while in this case these basal structures are absent.

The absence of the so-called phylogenetically young fiber systems is sufficiently explained by the lack of complete development of the suprasegmental neurones whose processes make up the bulk of such systems normally. There can be no doubt that the very slight attachment of the thalamic mass to the cerebral vesicle had much to do with the growth inhibition of the cortical neurones.

The presence of a well marked hippocampal formation while the rhinencephalon, as before defined, is entirely wanting, may be explained on mechanical grounds. Such a finding offers the

strongest evidence that the condition of the brain in cyclopia cannot represent an arrest of development at a phylogenetically early stage.

The observations in connection with the histological structure of the cortex, which will be more fully recorded elsewhere, have, I think, shown the value of a close investigation into the finer anatomy of the abnormal nervous system. It is in such cases as this that the key to many of the problems concerning the development of the normal brain may be found.

My conclusions can best be summarized as follows:

1. That the central nervous system in this case of cyclopia does not show any evidence that may be taken to indicate an arrest of its development at an early phylogenetic stage. From a study of Naegeli's paper, and from what I have seen in other cyclopic brains in my possession, I am led to the conclusion that any apparent indication of such a reversion is purely superficial in character and may be explained on mechanical grounds.

2. That the persistence of so-called phylogenetically old neurone systems in the brain, and the absence of phylogenetically young systems, is due solely to the absence during development of certain portions of the forebrain in the ventral region (mid-line), and to the interference with the mechanics of growth caused thereby.

3. That the condition of development of the primary forebrain will present a new mechanical problem in each case of cyclopia, depending upon the extent of the primary absence of tissue anlagen, and that the extent of this 'lesion' cannot always be judged by the condition of the cyclopic eye. In contrast to this, the condition of development in the brain stem in each case, provided no other malformations are present, will be very similar; and such abnormalities as are present will be due to the absence of fiber systems of the suprasegmental type.

4. That the condition of cortical development is such as to confirm the opinion already expressed by Bolton and Moyes (3), namely: that the sensory or afferent fibers to the cortex develop in all probability before the motor or efferent fibers from the cortex.

5. That the initial stimulus resulting in the differentiation of specialized efferent cortical neurones is probably dependent upon the arrival of these afferent fibers in the cortex.

6. That the condition of development in the cerebrum of this case offers further proof that the differentiation of the cortex into histologically distinct areas precedes the development of the convolitional pattern.

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¹ No attempt has been made here to prepare a complete bibliography of the literature dealing with cyclopia, for the great majority of the cases reported have no bearing upon the subject of this paper. A complete critical review of the literature up to 1872 has been made by Kundrat (12). For a short review of the subject of cyclopia up to 1897 and a statement of the various theories that have been held regarding the nature and cause of this malformation, reference may be made to Naegeli's paper (17). A somewhat more extensive historical survey of the subject, with a bibliography may be found in the thesis by Gravelotte (9). Most of the important contributions of recent date on this subject have been made by the application of experimental methods in the production of this malformation in lower forms. Some of the more important papers in this connection are cited, as well as some of the literature bearing upon the general subject of teratology. Papers dealing with the histological arrangement of the elements in the normal cortex, which have been consulted in connection with the study of the cortex in this case, are also referred to in this list.

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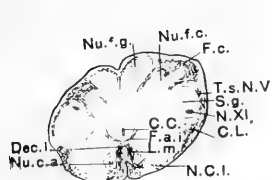
ABBREVIATIONS

FIGURES 24 TO 47

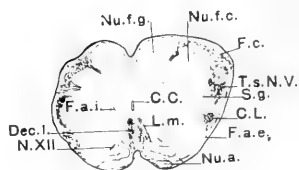
Figures 24 to 47 are drawn from a series of transverse sections through the cyclopiian brain stem, stained by a modified Weigert method followed by Upson's carmine. The drawings which are here reproduced at a magnification of $\times 2.5$, are all so arranged that the right and left sides of the sections correspond to the right and left sides of the page respectively. In examining this series it is well to bear in mind that in this brain stem two marked flexures persist. Thus for example, in figure 34, the midbrain and medulla appear in almost transverse section while the intervening pons is cut horizontally for the most part. An explanation of the abbreviations used in these figures will be found in the accompanying list.

<i>Aq.c.</i> , aquaeductus cerebri	<i>N.V.</i> ,	N. trigemini
<i>B.</i> , thalamic fibers that have wandered ventrad into the pia	<i>N.V.m.</i> ,	N. trigemini (motor)
<i>Br.c.</i> , brachium conjunctivum	<i>N.VI.</i> ,	N. abducentis
<i>C.c.</i> , canalis centralis	<i>N.VII.</i> ,	N. facialis
<i>C.hb.</i> , commissura habenularum	<i>N.VIII.</i> , <i>coch.</i>	N. cochleae
<i>C.isl.</i> , cell islands in second layer of cortex cerebri	<i>N.VIII.</i> , <i>vest.</i> ,	N. vestibuli
<i>C.i.</i> , colliculus inferior	<i>N.IX,X.</i> ,	N. glossopharyngei et vagi
<i>C.L.</i> , continuation into the medulla of the lateral column of the cord	<i>N.X.</i> ,	N. glossopharyngei et vagi
<i>Cor.cer.</i> , cortex cerebri	<i>N.XI.</i> ,	N. accessorii
<i>Cor.pin.</i> , corpus pineale	<i>N.XII.</i> ,	N. hypoglossi
<i>C.p.</i> , commissura posterior cerebri	<i>Nu.a.</i> , nucleus arcuatus	
<i>C.s.</i> , colliculus superior	<i>Nu.a.c.</i> , nucleus alae cinerae	
<i>C.t.</i> , corpus trapezoideum	<i>Nu.amb.</i> , nucleus ambiguus	
<i>D.Br.c.</i> , decussatio brachii conjunctivi	<i>Nu.c.a.</i> , nucleus of the anterior horn	
<i>Dec.l.</i> , decussatio lemniscorum	<i>Nu.c.i.</i> , nucleus colliculi inferioris	
<i>D.N.V.</i> , decussating tract of N. trigeminus	<i>Nu.coch.</i> , nucleus N. cochleae ventralis	
	<i>Nu.coch.d.</i> , nucleus N. cochleae dorsalis	
	<i>Nu.c.r.</i> , nucleus of restiform body	
<i>D.t.d.M.</i> , decussatio tegmenti dorsalis Meynerti	<i>Nu.d.</i> , nucleus dentatus	
<i>F.a.e.</i> , fibrae arcuatae externae	<i>Nu.emb.</i> , nucleus emboliformis	
<i>F.a.i.</i> , fibrae arcuatae internae	<i>Nu.f.c.</i> , nucleus funiculi cuneati	
<i>F.c.</i> , funiculus cuneatus	<i>Nu.f.g.</i> , nucleus funiculi gracilis	
<i>F.l.m.</i> , fasciculus longitudinalis medialis	<i>Nu.f.l.m.</i> , nucleus fasciculi longitudinalis medialis (Darkschewitsch)	
	<i>Nu.g.</i> , nucleus globosus	
<i>F.r.M.</i> , fasciculus retroflexus Meynerti	<i>Nu.h.b.</i> , nucleus habenulae	
<i>L.l.</i> , lemniscus lateralis	<i>Nu.l.l.</i> , nucleus lemnisci lateralis	
<i>L.m.</i> , lemniscus medialis	<i>Nu.lat.1,2,3.</i> , nuclei distinguished in the lateral portions of the thalamic mass	
<i>L.s.</i> , lemniscus superior	<i>Nu.lat.th.</i> , lateral thalamic nucleus	
<i>N.C.I.</i> , first cervical nerve	<i>Nu.med.th.</i> , medial thalamic nucleus	
<i>N.III.</i> ,		N. oculomotorii
<i>N.IV.</i> ,		N. trochlearis

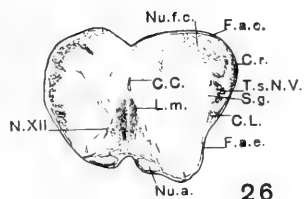
- Nu.m.N.V.*, nucleus motorius N. trigemini
Nu.N.coch., nucleus N. cochleae ventralis
Nu.N.coch.d., nucleus N. cochleae dorsalis
Nu.N.vest., nucleus N. vestibuli lateralis
Nu.N.III., nucleus N. oculomotorii
Nu.N.IV., nucleus N. trochlearis
Nu.N.VI., nucleus N. abducentis
Nu.N.VII., nucleus N. facialis
Nu.N.XII., nucleus N. hypoglossi
Nu.XII., nucleus N. hypoglossi
Nu.o.a.d., nucleus olivaris accessorius dorsalis
Nu.o.a.m., nucleus olivaris accessorius medialis
Nu.o.i., nucleus olivaris inferior
Nu.o.s., nucleus olivaris superior
Nu.Pon., nuclei pontis
Nu.r., nucleus ruber
P., thickened layer of pia enclosing midbrain and extraventricular portion of thalamus
Pl.ch.III., choroid plexus of third ventricle
Rad.th., thalamic radiations. (Ventral fibrillar area)
R.d.N.vest., radix descendens N. vestibuli
R.d.N.V., radix descendens (mesencephalica) N. trigemini
S.s., striae acusticae
S.a.p., stratum album profundum
S.g., substantia gelatinosa Rolandi
S.i.l., stratum interolivare lemnisci
St.z., stratum superficiale of cortex cerebri
T.s., tractus solitarius
T.th.I., attachment of the ependymal roof or cerebral vesicle over the anterior aspect of thalamus
T.th.III., taenia thalami
T.v.q., taenia ventriculi quarti
T.s.N.V., tractus spinalis N. trigemini
Vent.I., cavity of cerebral vesicle
Vent.III., ventriculus tertius
V.q., ventriculus quartus
X., ependymal diverticulum from aqueductus cerebri



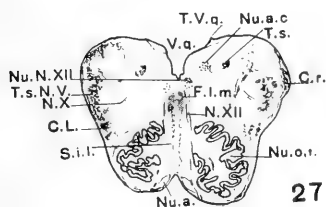
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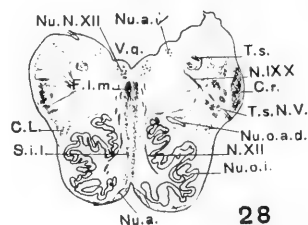
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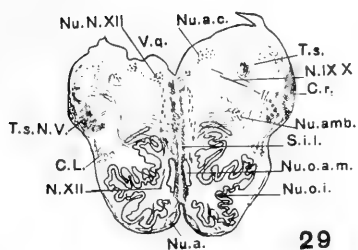
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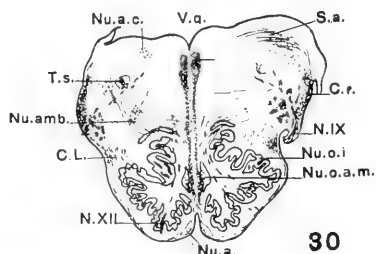
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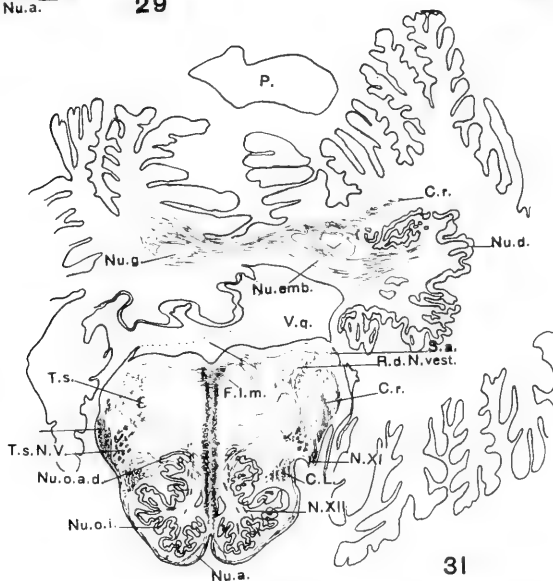
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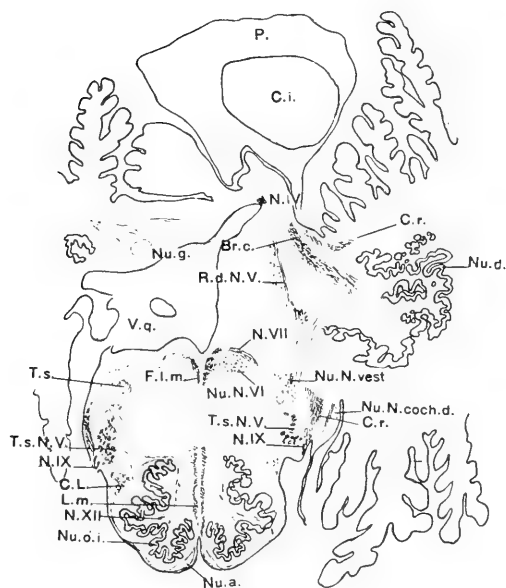
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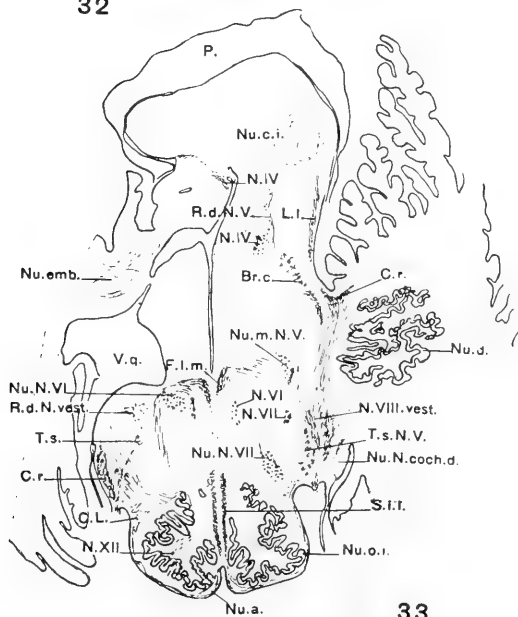
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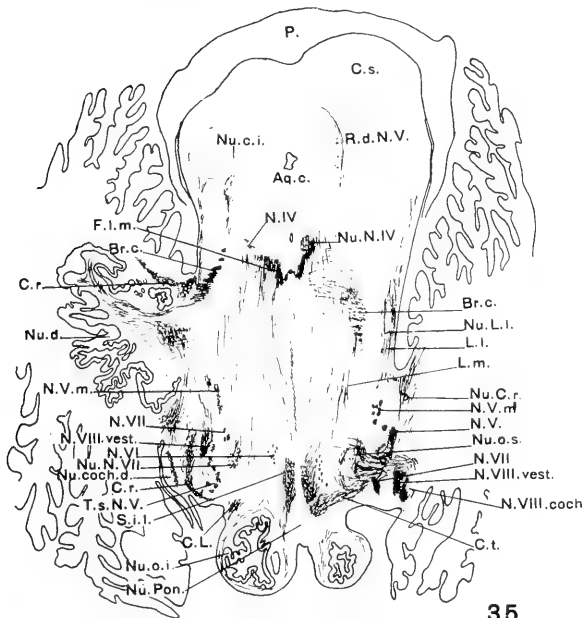
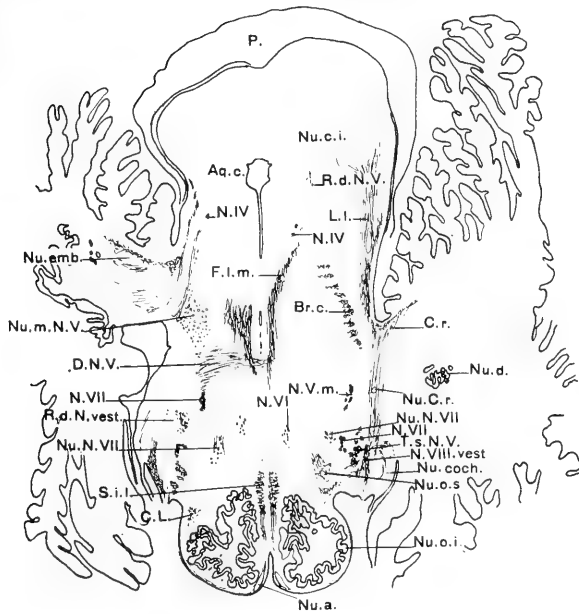
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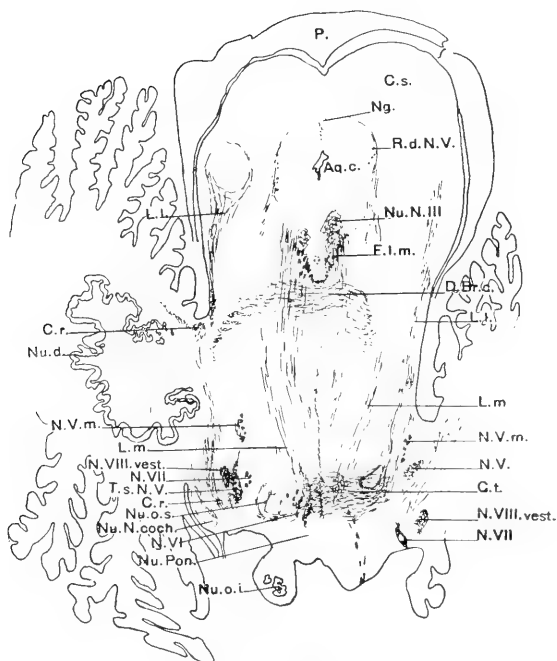


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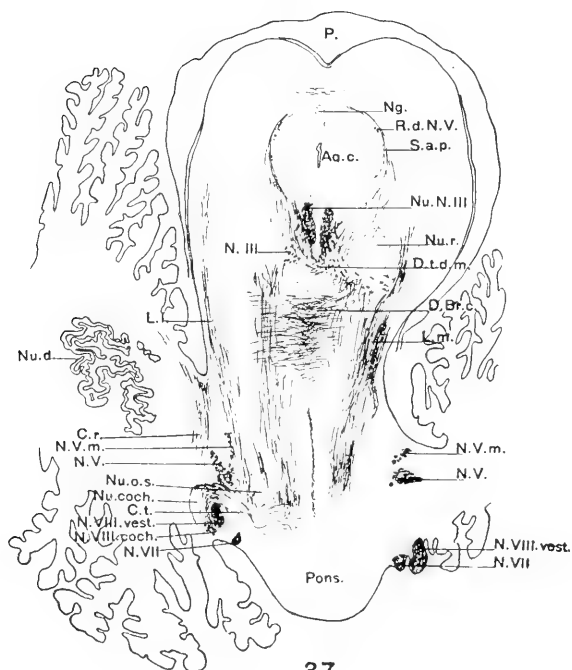


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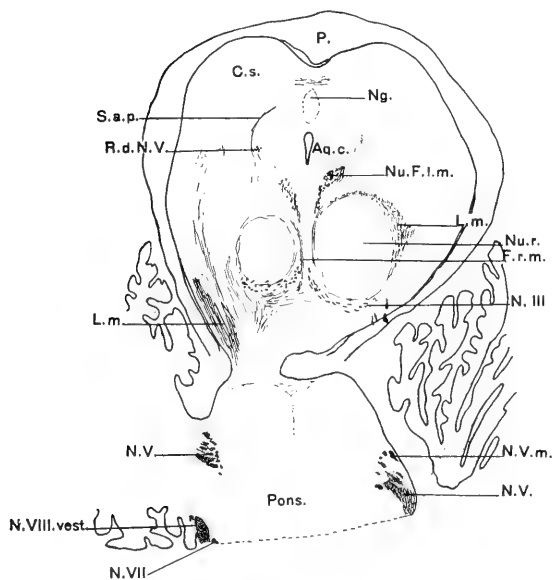




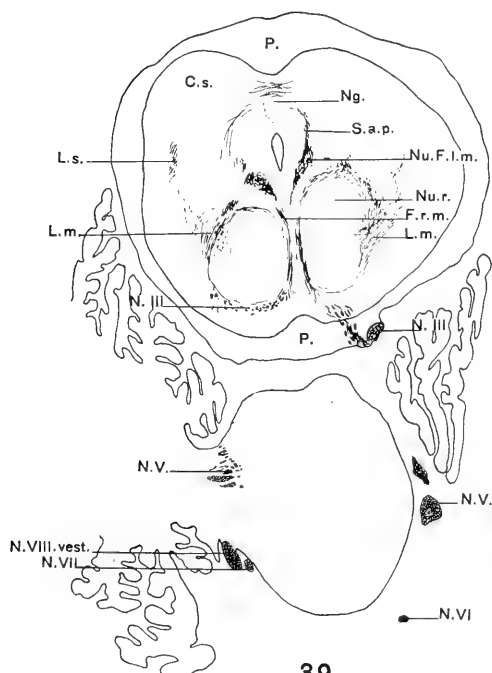
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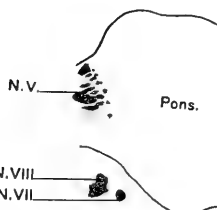
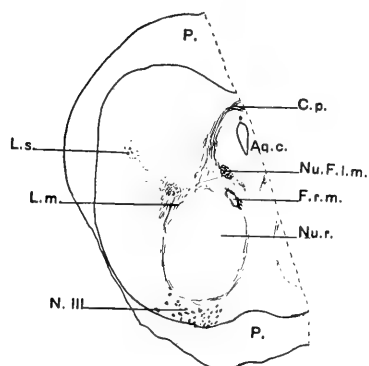
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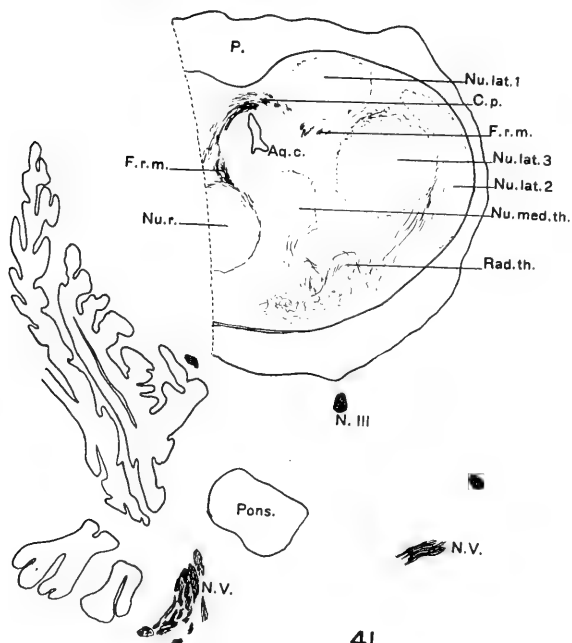
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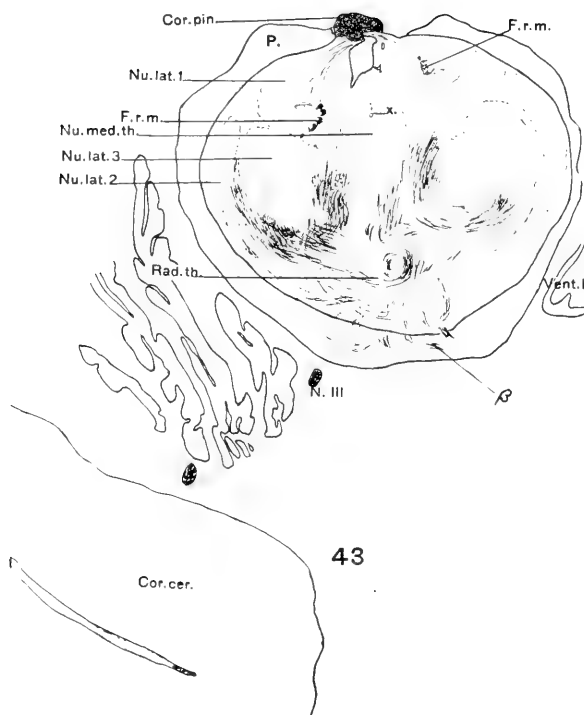
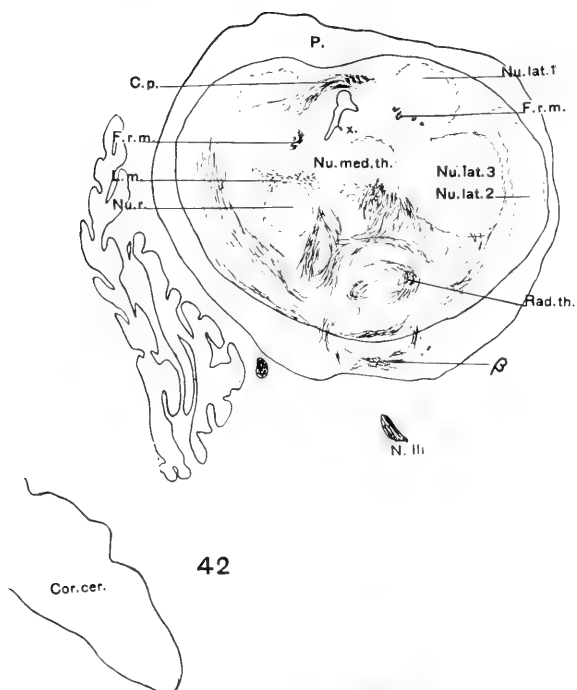
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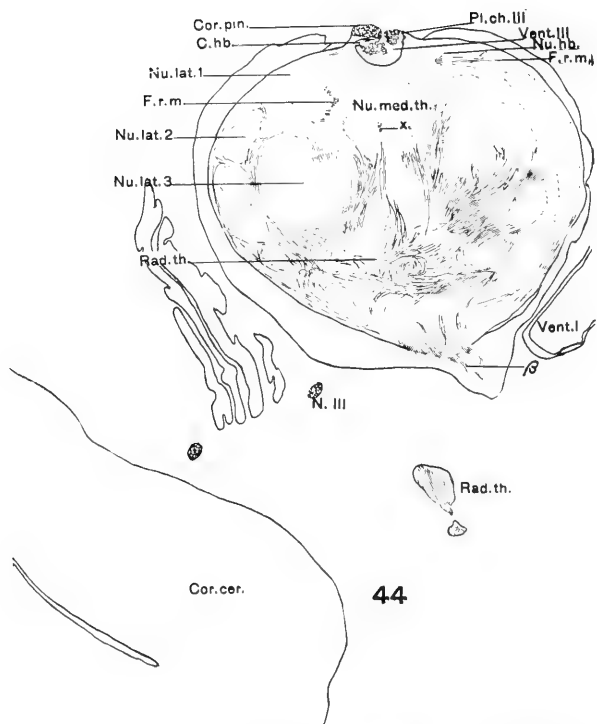


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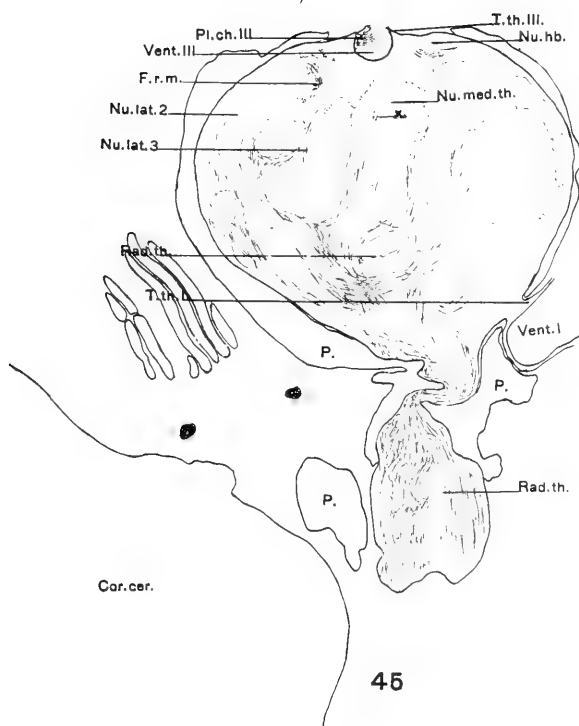


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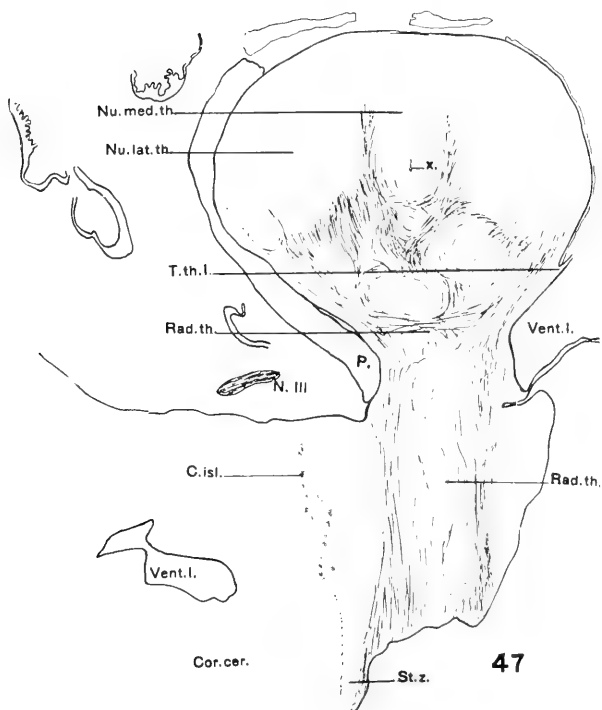
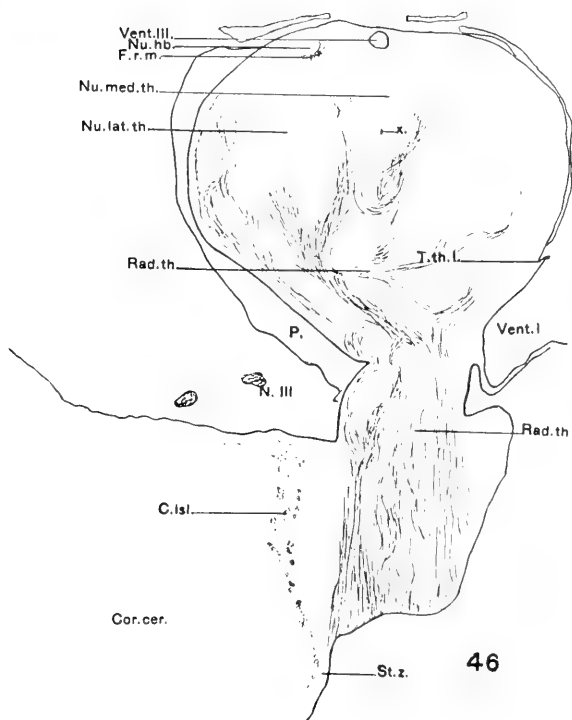


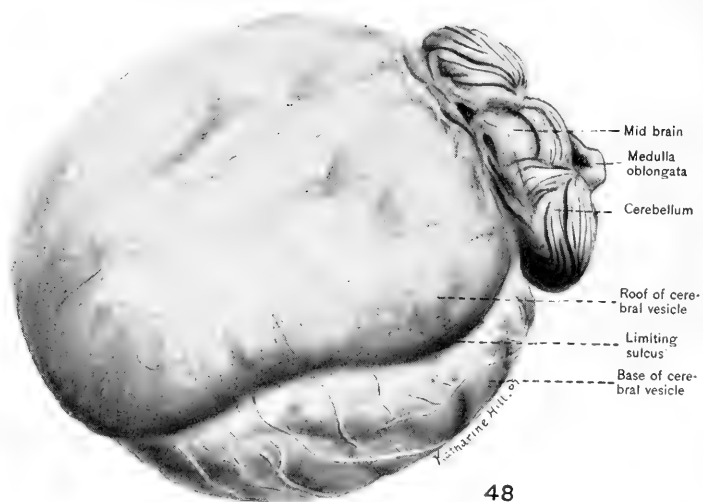


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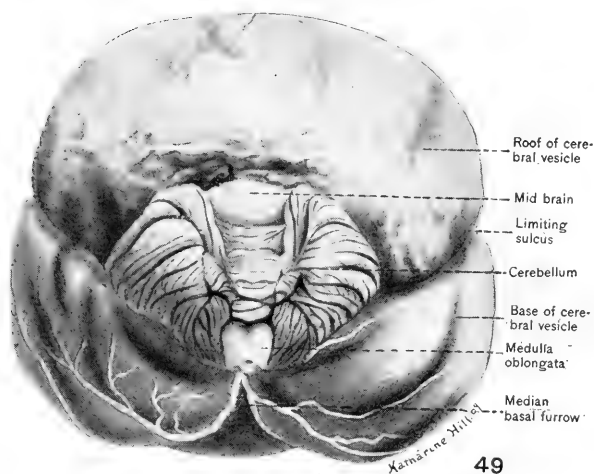


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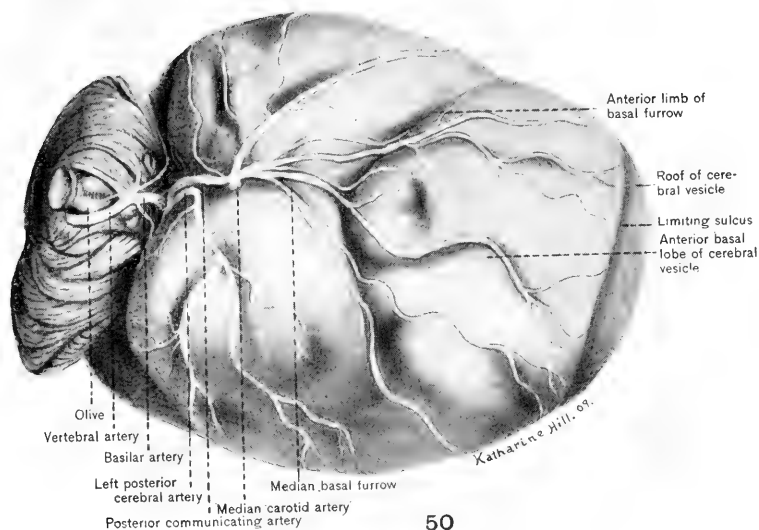
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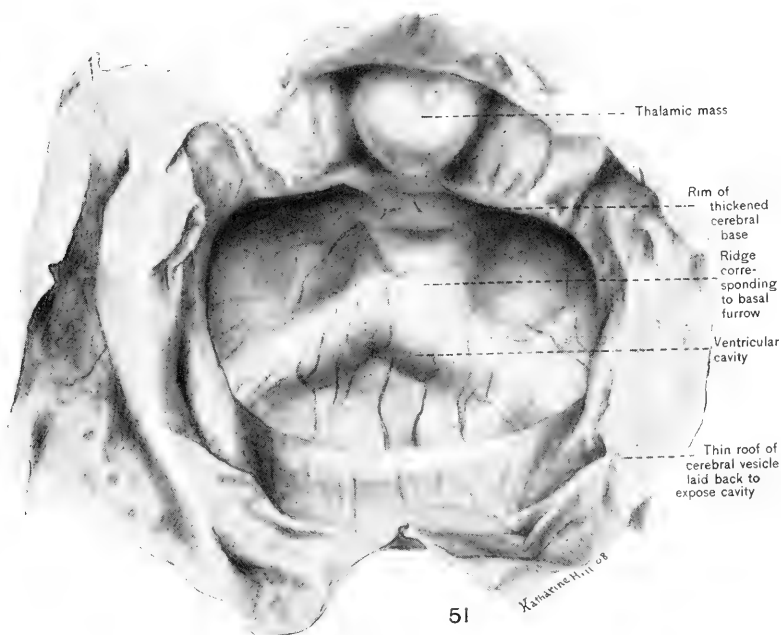
49

EXPLANATION OF FIGURES

- 48 Dorso-lateral view of entire brain. $\times \frac{2}{3}$.
 49 Posterior view of brain. $\times \frac{2}{3}$.



50



51

EXPLANATION OF FIGURES

50 Ventral view of brain. $\times \frac{2}{3}$.

51 Dorsal view of brain in front, with the thin roof of the forebrain vesicle opened up. $\times \frac{2}{3}$.

The Expressions of Emotion in the Pigeons. I. The Blond Ring-Dove (*Tur risorius*). By Wallace Craig. *Journal of Comparative Neurology and Psychology*, vol. xix, no. 1, April, 1909, pp. 29-82, with one plate.

ERRATA

I. In the musical records

Nos. 1, 3, and 31, and the score on page 33, the eighth-note with a line across its hook should have been made much smaller by the engraver; it is an acciacatura.

"Sve," wherever it occurs, applies to all the notes in that record.

No. 12, the first note is *mezzo forte*.

Nos. 10, 12, and 24, the sharps and flats represent a degree of sharpness or flatness less than a semitone. The runs are continuous, with very small intervals. In No. 12, the double-flatted e is but slightly lower than e flat. All this should have been explained in "Prefatory Remarks," p. 32-33.

Nos. 7, 8, 9, 10, 11, 14, and 31 should have no time signature and no division into bars.

No. 14, the c in the last bar is natural.

No. 17, the a in the last bar is natural.

No. 21, the crescendo sign over the second bar should be a diminuendo; in the last bar, the half-rest should be a whole-rest.

Nos. 22 and 23, omit the rest at end of each; also, in each, the syllables "go o" should be placed under the last two notes, and the "Sve" should be placed above the score.

Nos. 25, 26, 27, the single slur should be a double slur.

No. 33B, last bar, and No. 33C, last bar, the a is natural.

II. In the text

Page 43, line 5 from bottom, for *aspects* read *respects*.

Page 49, lines 12 and 8 from bottom, for "goo o" read "go o."

Page 51, line 7, for 16 read 46.

Page 56, line 18, for *legs* read *leg*.

Page 57, line 2 from bottom, for *fifteenth* read *sixteenth*.

line 1 from bottom, for *sixteenth* read *seventeenth*.

Page 58, line 1, for *twenty-fourth* read *twenty-fifth*.

line 2, for *twenty-third* read *twenty-fourth*.

line 4, for *thirtieth* read *thirty-first*.

Page 59, line 15, for *twelfth* read *thirteenth*.

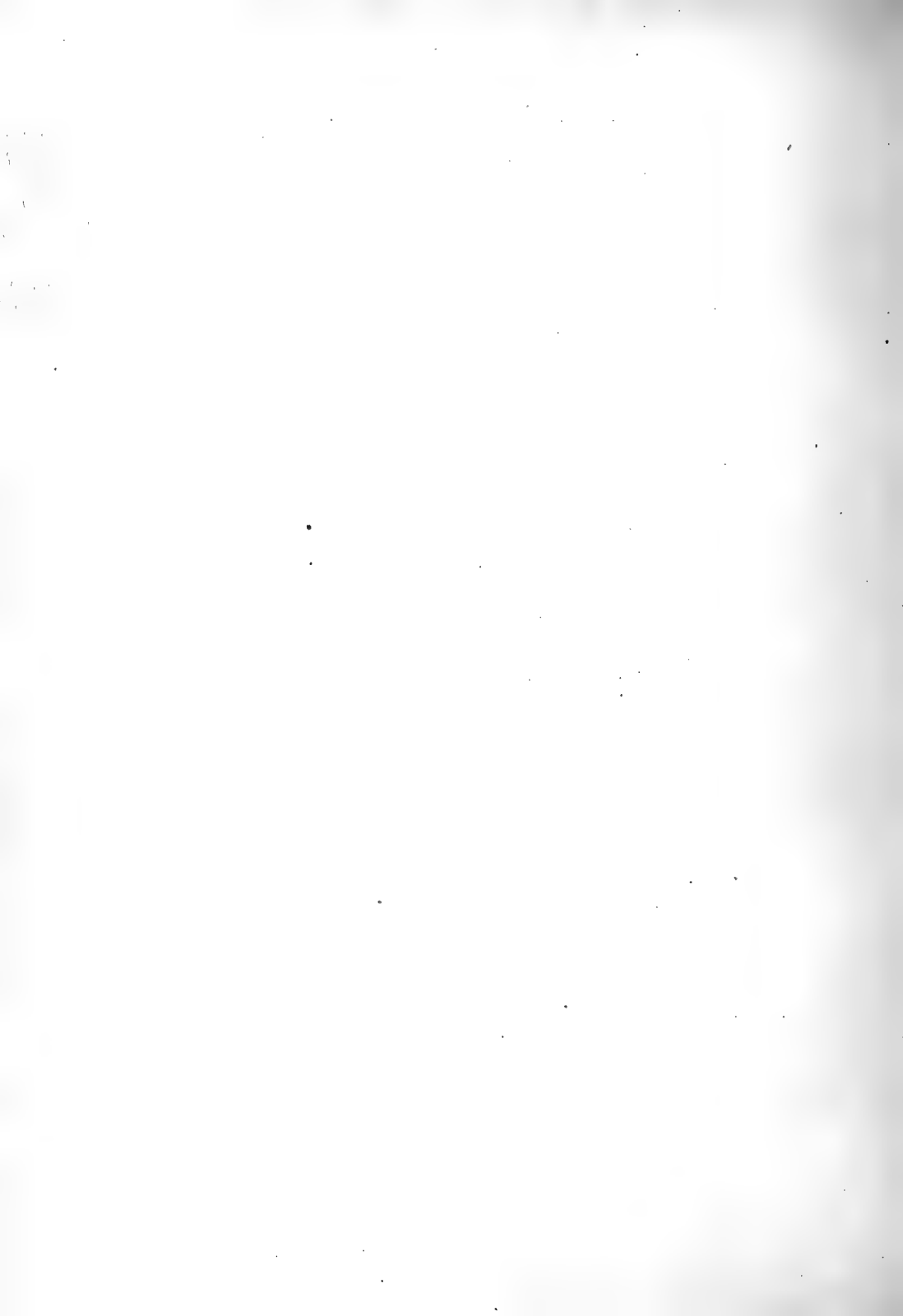
Page 66, in the table, first column, fifth item, for 9th to 14th read 10th to 14th sixth item, for 15th to 17th read 16th to 18th.

Page 73, line 4 from bottom, for *goes* read *coos*.

Page 77, line 3 to 2 from bottom, for *indiffer-cut* read *indiffer-ent*.

This table of ERRATA is printed and published in 1913.

Please insert this leaf at the beginning of the article referred to.



THE COURSE WITHIN THE SPINAL CORD OF THE NON-MEDULLATED FIBERS OF THE DORSAL ROOTS: A STUDY OF LISSAUER'S TRACT IN THE CAT

S. WALTER RANSON

From the Anatomical Laboratory of the Northwestern University Medical School

ELEVEN FIGURES

It has been shown that the small nerve cells of the spinal ganglia give rise to non-medullated axons, each of which divides dichotomously into a small non-medullated fiber running toward the periphery and an even smaller one centrally directed in the dorsal root. These non-medullated fibers can be followed for long distances in the nerve on the one hand, and through the dorsal roots to the spinal cord on the other. Just as the small cells of the ganglion far outnumber the large cells, so the non-medullated fibers of the nerve and the dorsal root far outnumber those which are medullated. Since these facts with the related literature have recently been discussed in detail (Ranson '11, '12) it will be unnecessary to repeat them at length in this place.

Partly with reference to this problem and partly with reference to the variations in the pyramidal tracts, we have recently been studying the spinal cord in a number of mammals. In looking over the accumulated material it became evident that the sections of the spinal cord of the cat were especially suited for the solution of the problem of the central course of the afferent spinal non-medullated fibers. In fact, the course of these fibers stands out with diagrammatic clearness in the pyridine-silver preparations of the spinal cord of the cat.

TECHNIQUE

The spinal cord of the cat forms very favorable material to which to apply the pyridine-silver technique. Preparations obtained from the cords of other laboratory animals (dogs, rabbits, guinea-pigs and rats) are not so perfect.

Under anaesthesia the animals were exsanguinated. The spinal cord was exposed and the ganglia on the C³, C⁷, T⁴, T⁸, T¹², L⁵, and S¹ spinal nerves were dissected out and left attached to their respective segments of the spinal cord. These segments with their associated roots and ganglia were then removed and freed from dura. Preparations were made by the Pal-Weigert as well as the pyridine-silver method. For the latter the pieces were not more than 5 mm. long, and after the usual treatment (Ranson '11), were imbedded in paraffin and cut into sections varying from 8 to 12 μ in thickness. Serial sections of a number of levels were mounted, to trace more in detail the entering bundles from the roots. The Pal-Weigert material was cut in celloidin into sections of 12 μ and 24 μ thickness. Usually sections of both thicknesses were cut from each segment. The technique of the silver stain has already been published (Ranson '11 and '12) and the Pal-Weigert method needs no explanation.

Structure of the substantia alba of the spinal cord

Cajal's silver method and its various modifications are known to give instructive preparations of the gray substance of the nervous system. In the preparations of the spinal cord of the cat it is superbly stained, showing the greatest detail in the pericellular fiber plexuses, and the endocellular fibrillar reticulum. It is, however, in the fiber columns of the cord that the pyridine-silver preparations present the most interesting differentiation. As in the peripheral nerves, the axons of the medullated fibers stain a light yellow and are surrounded by unstained rings of myelin; while the non-medullated axons are dark brown or black. Areas consisting chiefly of medullated fibers appear light yellow under low magnification because of the predominance of light yellow axons and unstained myelin. Other areas are lighter or

darker brown according to the number of dark brown non-medullated axons which they contain. Under higher magnification the non-medullated axons are sharply differentiated from the faintly yellow, granular neuroglia. The neuroglia fibers are not differentiated.

We wish briefly to call attention to those tracts which immediately surround the posterior cornu.

The cuneate and gracile fasciculi are composed very largely of medullated fibers (fig. 1). But in the neighborhood of the posterior commissure and posterior cornu there are numerous non-medullated fibers probably of endogenous origin. On the whole, however, these two fasciculi contain fewer non-medullated fibers than any other portion of the substantia alba, with one exception to be mentioned later. They are for this reason more lightly stained than is the anterior and most of the lateral funiculus. The number of non-medullated fibers in the fasciculus cuneatus and fasciculus gracilis is not sufficient to account for an upward continuation in these columns of the non-medullated fibers of the dorsal roots, and indicates that one must look elsewhere for their course within the cord.

The dorsal spino-cerebellar tract is also composed chiefly of medullated fibers, and is the most lightly stained of all the fiber columns of the cord. It is indicated in figure 1, *c*, as a light area contrasting sharply with the darker pyramidal tract (*d*) and the still darker tract of Lissauer (*b*). It is broadest near its posterior medial extremity where it is sharply marked off from the latter tract by a rather thick pial septum. It becomes gradually less distinct in an antero-lateral direction. Ventro-medially from this fasciculus and separated from it by no very sharp line is the pyramidal tract, *d*. The area occupied by the latter is somewhat rounded in outline and lies just lateral to the cervix and caput of the posterior cornu. Sharply outlined by its dark staining from the dorsal spino-cerebellar fasciculus, it is not so clearly separated from the remainder of the lateral funiculus. Next to Lissauer's tract the pyramidal tract is the darkest part of the substantia alba, and contains a very large number of non-medullated fibers. In comparison with the tract in the rat (Ranson '13),

the cat's pyramidal tract is much better medullated and contains in addition to non-medullated and small medullated axons also large axons with thick myelin sheaths. This is not the place, however, to enter into a discussion of the comparative histology of the pyramidal tract.

Lissauer's tract

Pal-Weigert preparations. Lissauer's tract occupies the apex of the posterior cornu, lying just dorsal to the substantia gelatinosa. It occupies the entire apex, reaching to the surface of the cord, except in the thoracic segments. Here the substantia gelatinosa, capped by the tract of Lissauer is at some distance from the surface; and the posterior part of the apex consists of a thin pial septum. Because of the light stain which the tract takes in Weigert preparations it is usually considered as a part of the posterior cornu, although it is admitted by all that it properly belongs with the longitudinal fiber columns of the cord.

In Pal-Weigert preparations it is seen to consist of rather sparsely arranged and uniformly fine medullated fibers. The spacing is uniform, each fiber being separated from its neighbors by a considerable interval. There is no grouping of these fibers into bundles nor are there any large spaces devoid of fibers. The fibers for the most part run vertically in the bundle, but some have an inclination forward toward the substantia gelatinosa. The obliquity of the fibers is most pronounced at the posterior extremity of the tract and in the neighborhood of entering root bundles. Starting in the region of an entering root bundle and passing forward toward the substantia gelatinosa, one can often see all gradations between horizontal fibers, and oblique fibers and again between oblique and vertical fibers. This would be in harmony with the generally accepted view that the medullated fibers of this tract are derived from the dorsal root, a view which has recently been called into question. In addition to the vertical and oblique fibers, just mentioned, one sees horizontal fibers of fine caliber, a few in each section running long distances through the tract to enter the substantia gelatinosa. These are prob-

ably in part fibers directly out of the dorsal roots which have not had a previous vertical course in the cord, and in part the horizontal continuation of the vertical fibers.

Although there is no septum separating the fasciculus cuneatus and entering root zone from Lissauer's tract, the border between the two is represented by a very sharp almost straight line; and the contrast between the closely packed large medullated fibers of the one and the more scattered fine medullated fibers of the other is very striking. On the lateral side the tract is separated from the lateral funiculus by a rather thick and very constant pial septum (not present in man). This pial septum never quite reaches the substantia gelatinosa; and ventral to this septum there is no sharp line of separation between this tract and the adjacent portion of the lateral funiculus. Lissauer's tract seems to extend laterally upon the dorsal surface of the substantia gelatinosa, its fine scattered medullated fibers gradually giving place to more thickly packed large ones.

This gradual transition between the ventral part of Lissauer's tract and the lateral funiculus attracted the attention of Leszlényi ('12) who made a very elaborate comparative study of Pal-Weigert preparations of the spinal cord of mammals, birds and reptiles. He saw many oblique fibers in the transition zone between the tract of Lissauer and the lateral funiculus; and concluded that many of the vertical fibers of Lissauer's tract come from the lateral funiculus through this transition zone. But the evidence which he presents in his paper, and the observations which I have been able to make on the cord of the cat, do not seem to me to show anything more than that there is an intermingling of the fibers of Lissauer's tract with those of the lateral funiculus ventral to the pial septum.

Leszlényi states that in all the cords he examined, with the possible exception of the human cord, the dorsal root fibers enter the posterior funiculus in such a way that one may be sure that no considerable number find their way into the tract of Lissauer. This seems to agree with the findings of Waldeyer ('88) on the cord of the gorilla, where few, if any, fibers could be traced from the dorsal roots into Lissauer's tract. Leszlényi finds, in all, four

kinds of medullated fibers in the tract of Lissauer: (1) fibers from Flechsig's ground bundle; (2) fibers which unite the posterior and lateral funiculi at the same level; (3) fibers out of the substantia gelatinosa which after a short course enter the gray substance again; (4) in man and many animals there are horizontal fibers which come from the dorsal roots and cross the tract of Lissauer to enter the substantia gelatinosa. In his opinion the dorsal roots contribute practically nothing to the vertical fibers of the tract.

This seems to be borne out, in part at least, by experimental and pathological data. There are a large number of papers, dealing with the course of the dorsal root fibers within the spinal cord, based on a study of Marchi preparations of the cord after lesions of the dorsal roots. Human cords, in which extensive degeneration of the dorsal roots has resulted from tumors, syphilis and other causes, have been studied as well as the cords of animals in which the roots have been divided. Most of these papers, although describing extensive degeneration in the posterior funiculus, make no mention of any degeneration in the tract of Lissauer. (See the papers of Darkschewitsch '96, Frölich '04, Kopeczynski '06, Margulies '96, Orr '06, Wallenberg '98 and Zappert '98.)

Nageotte ('03) states that the tract of Lissauer is composed of fine medullated fibers of endogenous origin. He maintains that they can not be derived from the dorsal roots, because in a case reported by him, in which a tumor involved all the spinal roots in the cauda equina up to and including the fourth lumbar, the fine fibers of the tract of Lissauer were intact. The presence of these intact fibers in this case shows conclusively that many, perhaps a majority, of the fine vertical medullated fibers of this tract are of endogenous origin. It can not be taken as conclusive proof that none of the fibers in this tract are derived from the dorsal roots.

Laignel-Lavastine ('08) studied the spinal cord in a case of syphilis involving the cauda equina. The Marchi stain showed a very few degenerating fibers in Lissauer's tract, and the Weigert stain showed the vast majority of the fibers in this tract to be

intact. He admits the contention of Nageotte that such degeneration as he finds may be tertiary, but insists that one cannot be certain that the tract does not contain some fibers from the posterior roots.

Sibelius ('05), who studied three cases in which the cauda equina was involved, found some degeneration in Lissauer's tract which he considers as the direct result of the root lesion. He explains the negative findings of Nageotte by assuming that the fine horizontal fibers of Lissauer's tract had disappeared and that the author had failed to notice their absence. This explanation is tantamount to an admission that the proportion of dorsal root fibers in the tract is small and that they are chiefly horizontal.

Sottas ('93) and Collier and Buzzard ('03) find a limited amount of degeneration in Lissauer's tract after dorsal root lesion.

The evidence seems to show that the medullated fibers in the tract of Lissauer are in part endogenous and in part exogenous and that the endogenous fibers predominate. We shall return to this question again in discussing the entrance of the dorsal root fibers into the spinal cord.

Pyridine-silver preparations. In pyridine-silver preparations the tract of Lissauer is stained very dark and is even more sharply outlined from the rest of the cord than in the Pal-Weigert preparations. It is seen to consist of vertically or obliquely coursing axons of the smallest diameter (fig. 2, *b*). These are stained a brownish black and are very sharply differentiated from the almost colorless background. They are very closely set together, although there are scattered among them a few medium sized yellowish brown axons which correspond in number and arrangement to the medullated fibers seen in the Pal-Weigert preparations. When compared with Pal-Weigert preparations of the same segment of the cord the contrast is very striking. In the latter the tract of Lissauer is very lightly stained and is seen to consist of rather sparsely scattered fine medullated fibers. A glance at the two preparations is sufficient to show that the number of axons in the one is several times greater than the number of myelin sheaths in the other. Lissauer's tract consists then in part of small medullated fibers but its chief and charac-

teristic content is an enormous number of fine vertically coursing non-medullated fibers.

Before taking up the account of the course taken by the dorsal root fibers as they enter the cord it will be desirable to call attention to the variations in shape and topography of the tract of Lissauer at different levels in the cat's cord. In the cervical region as illustrated by the seventh cervical segment (fig. 1) the tract is long and narrow, since the substantia gelatinosa is at some distance from the surface of the cord and the tract fills the entire apex of the posterior cornu. Throughout the entire thoracic region of the cord (fig. 3, T. 8) the posterior cornu is not well developed; the substantia gelatinosa is placed a long distance from the surface of the cord; and the cone-shaped Lissauer's tract which caps it is also some distance removed from the surface. The apex of the cone is connected with the surface by a septum containing few fibers; occasionally isolated portions of Lissauer's tract are seen along the medial side of this septum. In the lumbar cord (fig. 4, L. 5) the substantia gelatinosa has approached the surface; and the tract of Lissauer, which fills the interval between it and the surface has become short and wide. In the sacral region (fig. 5, S. 1) the substantia gelatinosa has flattened out laterally and the tract of Lissauer occupies a short but wide interval between it and the periphery of the cord.

The size of the tract varies somewhat from level to level and seems to be roughly in proportion to the size of the nerve roots entering at that and adjacent levels. The first sacral segment seems to be an exception to this rule. Although its entering rootlets are larger, it presents a Lissauer's tract somewhat smaller than that in the fifth lumbar segment. These facts would be easily explained if we assume that the majority of the non-medullated fibers in the tract are short ascending fibers from the dorsal roots. The small rootlets of the lower sacral segments would then contribute fewer ascending fibers to the tract in the first sacral segment, than would be contributed by the large rootlets of the upper sacral and last two lumbar nerves to the tract in the fifth lumbar segment. The ascending fibers must be relatively short, however. Otherwise there would be a steady increase

in size from lower to high levels in the cord. But the tract seems rather to be proportional in size to the entering rootlets; and instead of a steady increase in size in an ascending direction, there is a marked decrease in going from the lower lumbar into the thoracic cord and again in passing from the lower cervical to the upper cervical segments. These facts indicate that the majority of the non-medullated fibers are short ascending fibers and in the next section we will show that many, probably a great majority, are derived from the dorsal roots.

Entrance of the dorsal roots into the spinal cord

Pal-Weigert preparations. In 1885 Lissauer observed that fine medullated fibers grouped themselves on the lateral side of an entering rootlet and turning lateralward separated themselves from the remainder of the rootlet to enter the apex of the posterior horn, where they turned to run vertically in the tract which now bears his name. Similar observations were made by Bechterew ('86), and have formed the basis of the standard text-book accounts of this tract. It is an easy matter to confirm these observations in Pal-Weigert preparations of the cat's cord, especially in the case of the larger cervical and sacral roots. There can be no doubt that in the cat medullated fibers enter the tract of Lissauer from the dorsal roots in considerable number, and in exactly the manner described by Lissauer and Bechterew. There can also be no doubt, on the basis of the pathological and experimental evidence presented in preceding paragraphs, that these medullated fibers from the dorsal root do not constitute all or even the majority of the medullated fibers in this tract.

Pyridine-silver preparations. To the medial side of the tract of Lissauer is the entering root zone. In this region the medullated fibers, which have just entered the cord, can be seen running more or less obliquely (fig. 10, *a*). The fibers seen here are for the most part large medullated ones; and, when one remembers the large number of non-medullated fibers seen in the dorsal root, one is impressed with the scarcity of these fibers in the entering root zone. Somewhat further medialward large numbers of fine

collaterals are given off from the medullated fibers and take a more or less oblique course toward the posterior horn. But these, for the most part, take a much lighter stain than the non-medullated fibers. Occasionally medium sized bundles of non-medullated fibers are seen in the entering root zone as at *e* in figure 10, but such bundles usually have a direction nearly at right angles to the medullated fibers, and are making their way toward the tract of Lissauer. We shall see that the majority of non-medullated fibers separate themselves from the medullated just before the entrance into the cord. The scattered non-medullated fibers as well as the bundles of such fibers in the entering root zone, were delayed in their separation from the medullated fibers; but most of them finally find their way into the tract of Lissauer. It is possible, however, that a few of the non-medullated fibers pass medially to the tract of Lissauer and enter the fasciculus cuneatus.

A dorsal root as it enters the cord is broken up into a large number of fila radicularia or rootlets. As each rootlet enters the cord it is surrounded and constricted by an encircling band of pia. Shortly before the rootlet reaches this constricting band, the non-medullated fibers, which nearer the ganglion have been distributed quite uniformly throughout the root, separate out from the medullated ones and come to lie either at the periphery of the radicles or along septa which divide the radicles into smaller bundles. In this way large flat bundles of non-medullated fibers are formed; often a thin layer of such fibers is seen making a complete tubular sheath at the periphery of the radicle. In serial sections it is possible to trace these compact bundles into the cord and see that they enter Lissauer's tract. It is this early separation of these fibers from the main mass of the radicle that causes the entering root zone to be composed almost entirely of medullated fibers.

We will now take several typical instances and show how these fibers can be traced into Lissauer's tract. In the first sacral segment (figs. 5, 6 and 7) the radicles are of good size and are divided by connective tissue septa, running in a general antero-posterior direction, into smaller fascicles which are displaced

medially as one after another enters the cord. Figure 5 is a diagrammatic representation of the level from which figures 6 and 7 were taken. At *a* is indicated the tract of Lissauer; *b* and *c* represent connective tissue septa. In the high power drawings the same lettering has been used. In figure 6 one sees that most of the non-medullated fibers have separated out from among the medullated and arranged themselves along the septa *b* and *c*. At *d* and *d'* are seen bundles of non-medullated fibers arranged along the inner border and the medial part of the posterior border of the radicle. The fibers in bundle *d* can be traced at this level into Lissauer's tract. The fibers in *d'* can be traced along the surface of the cord for a short distance and then turn ventrally into the same tract. Bundle *c* is composed of fibers which have separated out from the two root fascicles between which it lies. It is composed of two layers of fibers arranged one on each side of a connective tissue septum. If one follow this bundle upward in the serial sections its fibers are seen to turn ventrally and run into the tract of Lissauer (fig. 7, *c*). These fibers along the connective tissue septa of the roots are in size and staining reaction exactly like the non-medullated fibers which farther distally are scattered uniformly through the roots. They are also identical in size and staining reaction with the non-medullated fibers of the tract of Lissauer into which they have just been followed. It seems that a clearer demonstration of the fate of the non-medullated fibers of the dorsal roots could scarcely be desired.

In the fifth lumbar segment of the cat's cord the entering radicles are smaller. Only when they are covered by a thick coat of connective tissue and bound down tightly to the surface of the cord are the physical conditions satisfactory for the impregnation of the non-medullated fibers. Figures 4 and 8 show such a rootlet entering the cord. Notice that its fibers pass through Lissauer's tract and separate off a small dorso-median portion from the main tract. This unites again with the rest of the tract above and below the entering root bundle. The constricting ring at the entrance of a radicle into the cord is well seen in figure 8. Points *a* and *c* are joined in the thickness of this section and the next by an arched band of connective tissue. The

projecting points and this band form part of the constricting ring. Upon the surface of this band and separating it from the medullated fibers of the bundle can be seen a layer of closely packed non-medullated fibers, *b*. They can be followed for only a short distance at *b* as they arch over the constricting band but between *b* and *c* they can be traced over this band into Lissauer's tract. Along the ventral surface (*d*) of the extra-medullary part of the bundle and along the connective tissue septum (*e*) separating this from the next root bundle the non-medullated fibers have accumulated. Along the line *a*, *b*, *c*, *d*, *e*, there is indicated a peripheral layer of non-medullated fibers which a little more than half surrounds the bundle of entering medullated fibers. Whether a similar layer is present on the dorso-medial surface of the radicle it is impossible to say, since it is not likely that the fibers would take the stain in this superficial position if they were present.

The general principles which govern the entrance of the non-medullated fibers into the cord are well illustrated by the radicle from the seventh cervical dorsal root which is shown at three levels in figures 9, 10 and 11. The lettering in these three figures is the same as in figure 1 from the same segment and to which reference should be made for orientation. Figure 9 is from a section just above the level of entrance of the radicle in question. The medullated fibers of this rootlet are seen cut obliquely at *a*, and at *b* is indicated the tract of Lissauer. At 1 is seen a bundle of non-medullated fibers which are arching ventro-laterally over the obliquely coursing medullated fibers, *a*, of the entering rootlet. Dorso-laterally bundle 1 is continuous with bundle 2 of more vertically running fibers. A study of serial sections shows that bundle 1 is derived from two sources. First there are upon the upper surface of bundle *a*, as it enters the cord, a large number of non-medullated fibers which turn upward and then ventro-laterally to enter into the formation of bundle 1. A few of the uppermost of these are seen at 3. The second group of fibers entering into bundle 1 are derived from bundle 2.

Tracing the same structures downward through the series we find them arranged as in figure 10. Here bundle *a*, composed

chiefly of medullated fibers, is seen entering the cord. At 4 a bundle of non-medullated fibers can be seen running over the constricting ring to pass directly into the tract of Lissauer on entering the cord. At 5 are seen a few non-medullated fibers gathering along a line which represents the beginning of a line of separation between two fascicles of the root. Bundle 2 is larger here than in the preceding section and is placed between bundle *a* and another bundle more dorsally situated. It is composed of fibers which have separated out from these two bundles and especially from bundle *a*, and, instead of running directly across the bundle of medullated fibers to reach Lissauer's tract, have turned upward or downward on the dorsal surface of bundle *a*. In this way a vertical bundle of large size is formed, which traced upward is seen to arch ventro-laterally over the upper surface of bundle *a* to form part of bundle 1, figure 9, and so to reach the tract of Lissauer. Traced downward, the descending fibers which enter into its composition are seen to turn ventro-laterally below bundle *a* to run into Lissauer's tract (fig. 11). Here they form part of a rather wide band of fibers on the medial and under surface of bundle *a*. A part of this band is seen at 6, figure 11. But the band is much wider than is indicated in the figure. In the succeeding sections the band is seen to underlie bundle *a* to the very edge of the cord. This band is composed in part of fibers derived from bundle 2, figure 10, and in part from fibers entering the cord as a layer upon the under surface of bundle *a*.

It thus appears that the non-medullated fibers separate out from the bundles of entering root fibers. They occupy the periphery of the bundles as these enter the cord and then take the route of least resistance to reach Lissauer's tract. In the case of the fibers on the dorso-medial surface of such entering root bundles this path of least resistance is usually around the bundle of entering medullated fibers rather than through it. For this reason these fibers form bundles arching over or under the bundle of medullated fibers. For those non-medullated fibers occupying the ventro-lateral portion of the surface of the entering dorsal root bundle the path to Lissauer's tract is direct (fig. 10, 4). In

the case of the first sacral segment the non-medullated fibers separate out along the septa separating the fascicles of the rootlet, and run forward along these septa into the underlying Lissauer's tract before the rest of the bundle has entered the cord.

The non-medullated fibers of the dorsal root, then, enter the tract of Lissauer, of which they form the chief and characteristic part. They run for short distances in this tract chiefly in an ascending direction and then probably pass forward into the substantia gelatinosa. The close relation of Lissauer's tract to this peculiar substance which caps the posterior horn, the fact that fibers can be seen passing from one into the other, and the fact that there is no other apparent outlet for the fibers of Lissauer's tract indicate that the substantia gelatinosa is the probable nucleus of reception of these non-medullated fibers. The fact that the substantia gelatinosa contains a large number of very small nerve cells and many non-medullated nerve fibers is of interest in connection with the probable relation of the two structures. Experimental evidence is needed, however, to prove this relation conclusively.

So far as the function of the non-medullated fibers is concerned, their course within the cord shows that they can have little or nothing to do with the afferent impulses received from muscles and joints which travel up the posterior funiculus. This does not necessarily include muscle and joint pain. Their early termination within the gray substance would agree with the course of the sensations of pain and temperature and probably also with that of touch. But there are, of course, no data on which one would care to hazard a guess as to their function, beyond the statement that they can have little or nothing to do with those sensations which are known to travel directly upward in the posterior funiculus.

SUMMARY

1. The small cells of the spinal ganglion give rise to non-medullated fibers whose centrally directed branches form the non-medullated fibers of the dorsal roots.

2. The non-medullated fibers of the dorsal roots can be traced with diagrammatic clearness into Lissauer's tract.

3. The tract of Lissauer contains rather sparsely arranged fine medullated fibers which are in part derived from the dorsal roots but are in greater part of endogenous origin.

4. The tract of Lissauer contains a very great number of fine non-medullated axons, at least the great majority of which are derived from the non-medullated fibers of the dorsal roots.

5. It is probable that the substantia gelatinosa is the nucleus of reception for the non-medullated fibers.

6. It seems clear that the non-medullated fibers have little or nothing to do with the transmission of the afferent impulses from the muscles and joints, at least with such part of these impulses as are transmitted upward in the posterior funiculi.

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PLATE 1

EXPLANATION OF FIGURE

The drawings were made with the aid of a camera lucida from pyridine-silver preparations of the spinal cord of the cat.

1 From the seventh cervical segment. The substantia grisea presents a dense mass of interlacing fibers. The nerve cells are indicated in solid black. Capping the posterior cornu is the lightly staining substantia gelatinosa. More dorsally is seen the darkly staining tract of Lissauer, *b*. At *a*, is seen the obliquely cut medullated fibers of the entering dorsal root in the fasciculus cuneatus. At *c*, is represented the dorsal spino-cerebellar tract, and at *d*, the pyramidal tract. The differentiation of the fiber columns of the cord shown in the drawing is due to the varying proportion of non-medullated fibers which they contain. These are most abundant in the tract of Lissauer. The pyramidal tract contains the next greatest number, and the dorsal spino-cerebellar tract the fewest. $\times 23$.

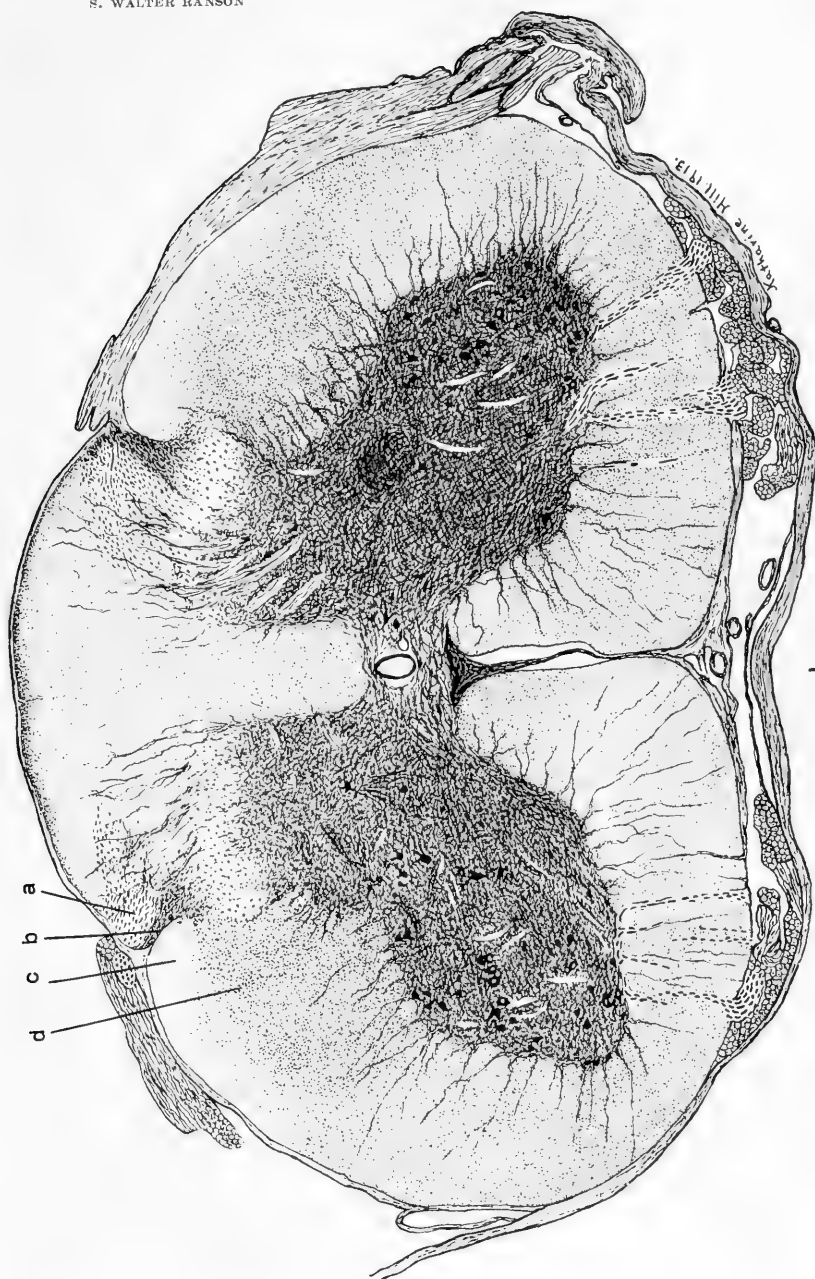


PLATE 2

EXPLANATION OF FIGURES

2 From the seventh cervical segment. A narrow strip at right angles to the apex of the posterior cornu: *a*, fasciculus cuneatus; *b*, Lissauer's tract; *c*, the dorsal spino-cerebellar tract. The medium sized and large lightly stained axons are medullated, the fine darkly stained ones non-medullated. The latter are very numerous and closely packed together in the tract of Lissauer. $\times 648$.

3 Diagrammatic representation of the eighth thoracic segment, showing the shape and position of Lissauer's tract. $\times 17$.

4 Diagrammatic representation of the fifth lumbar segment, showing the shape and position of Lissauer's tract. $\times 17$.

5 Diagrammatic representation of the first sacral segment, showing the shape and position of Lissauer's tract. *a*, Lissauer's tract, *b* and *c*, connective tissue septa dividing the entering root bundle into smaller fascicles. Bundles of non-medullated fibers are grouped along these septa. Other bundles of non-medullated fibers are seen at *d* and *d'*. $\times 17$.

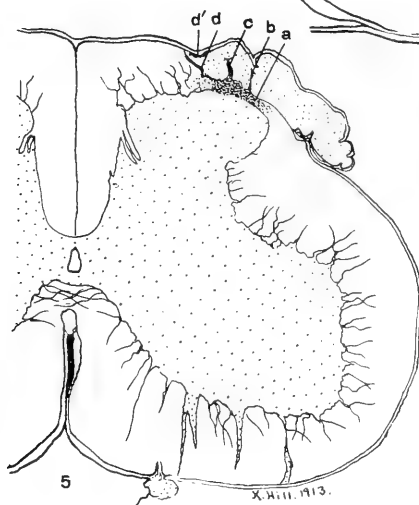
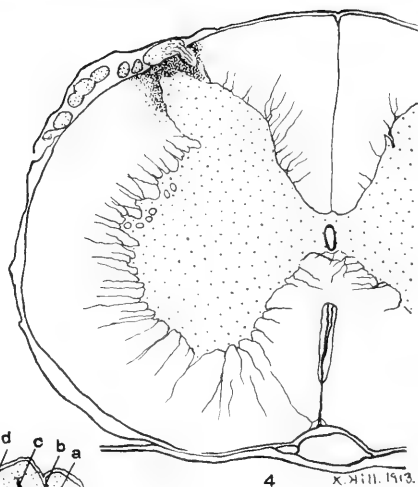
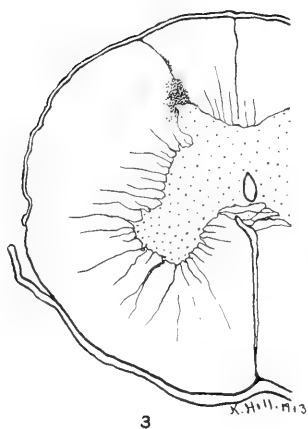
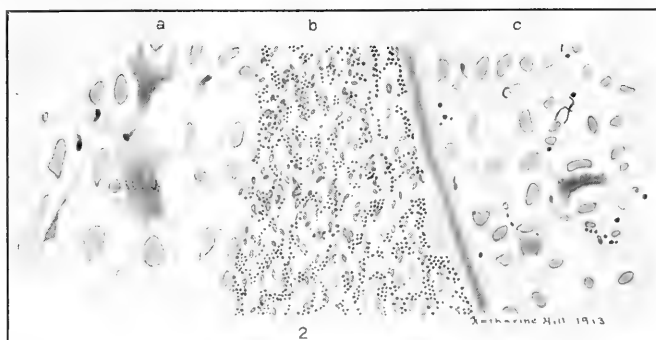


PLATE 3

EXPLANATION OF FIGURES

6 Lissauer's tract and entering dorsal root in the first sacral segment. Lettering is the same as in figure 5, which gives the topography of the high power drawing. Non-medullated fibers are separating out along the borders of the entering radicle and along the connective tissue septa which separate it into fascicles. At *d* non-medullated fibers are seen running forward into Lissauer's tract. $\times 100$.

7 Same area as represented in figure 6 but about 50μ farther cephalad. Lettering the same as in figures 5 and 6. The non-medullated fibers of the dorsal root, which have separated out along the connective tissue septum *c*, are seen running forward into the tract of Lissauer, *a*. $\times 100$.

8 Lissauer's tract and entering dorsal root in the fifth lumbar segment. For topography see figure 4. At *a*, *b*, *c*, is seen a part of an encircling band of pia which surrounds and constricts the entering radicle. Upon the surface of this band a layer of non-medullated fibers is seen entering the cord. At *d*, and *e*, are seen thin layers of non-medullated fibers at the periphery of the radicle and along the septum separating the radicle into two fascicles. $\times 100$.

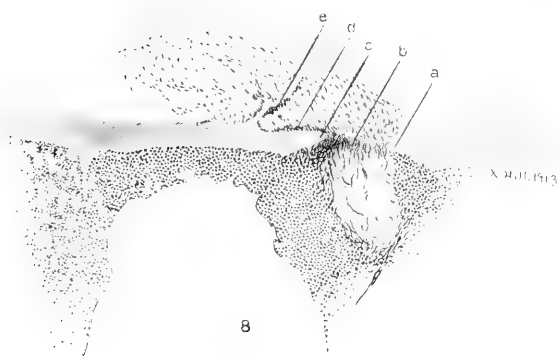
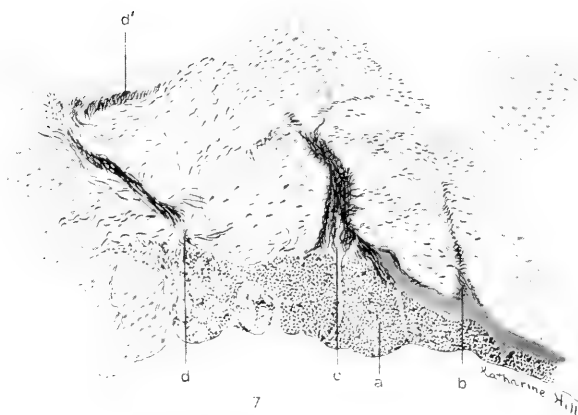
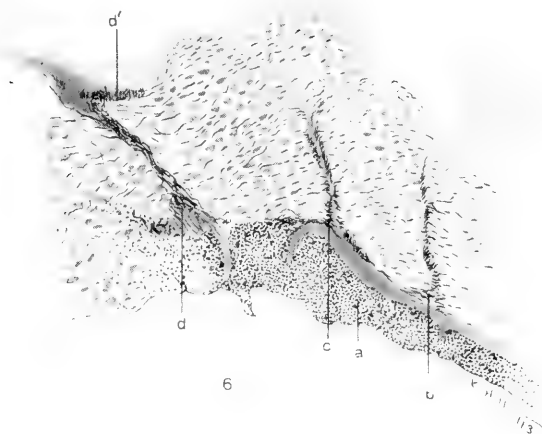
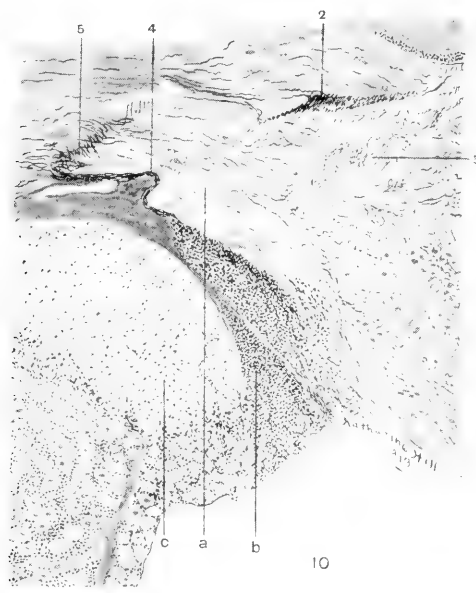
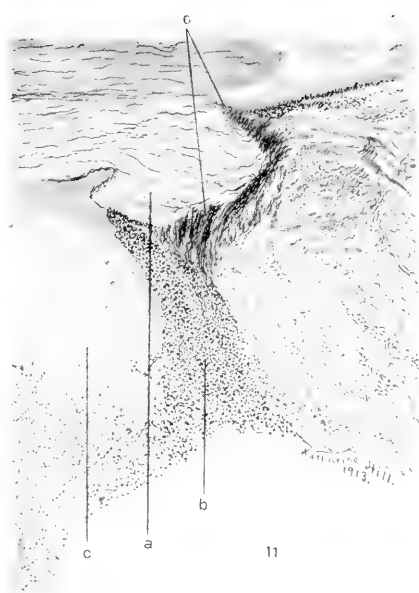
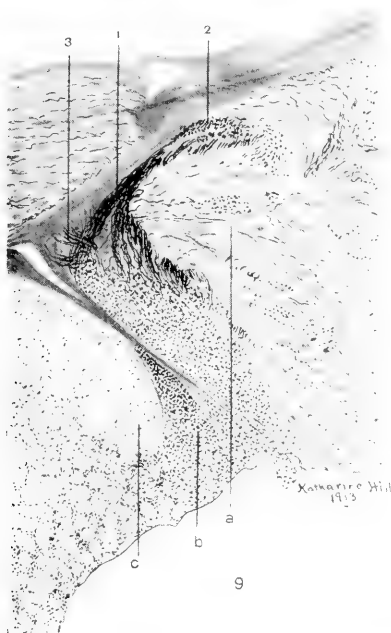


PLATE 4

EXPLANATION OF FIGURES

9, 10 and 11 Three sections from a series through an entering radicle of the seventh cervical dorsal root. For topography see figure 1. Figure 9 represents a level just above the entrance of the radicle (*a*) into the cord, figure 10, a level through the middle of the entering radicle, and figure 11, a level near its lower margin. The lettering is the same as that in figure 1. At 1, 2, 3, 4, 5 and 6 are indicated bundles of non-medullated fibers which can all be traced into Lissauer's tract. $\times 82$.



THE EFFECTS OF FORMALDEHYDE ON THE BRAIN OF THE ALBINO RAT

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TEN CHARTS

Although formaldehyde was discovered in 1863, it was not until thirty years later that Blum ('93) and Hermann ('93), working independently, found that an aqueous solution of this substance is an excellent medium for preserving and hardening various animal tissues. Owing to its many admirable properties and to its relative cheapness, 'formalin' (a commercial 40 per cent solution of formaldehyde) soon became extensively employed as a fixing and preserving reagent for entire brains as well as for other large pieces of tissue, being used either in a 5 to 15 per cent aqueous solution or combined with other substances such as alcohol, alum or salt.

In an extensive series of experiments dealing with the effects of various formalin solutions on the weight of the brains of man, of sheep and of various other mammals, Hrdlicka ('06) showed that the size of the brain, the age of the animal from which the brain was taken and the strength of the solution used were all factors that influenced the weight and volume changes in the brain to a very considerable extent. Hrdlicka did not, however, ascertain the relative importance of these various factors, nor did he make any study of the histological effects produced in the brain tissue by formaldehyde solutions. At the present time formalin is commonly used in laboratories and museums as a fixative and also as a preservative for the brains of man and of other mammals. It has seemed worth while, therefore, to make a careful study of the changes produced by this substance, acting under different conditions, on a series of brains from animals of known ages from birth to maturity. Experiments of this kind

ought to define the limits of the use of formalin as a brain preservative and to indicate when and how this substance can be used to the greatest advantage in neurological work. The present paper records the results of such a series of experiments made on brains of the albino rat (*Mus norvegicus albinus*). As a 4 per cent solution of formaldehyde (10 per cent formalin) is the one that the experience of many investigators has shown is the best for brain preservation as well as for general histological work, a solution of this strength was the only one used in this series of investigations.

The technique employed in all the experiments was as follows: Animals of known ages were killed with ether, and their body lengths and body weights recorded. The brain, with its meninges intact, was removed as soon as possible after the death of the animal, being cut from the cord at the tip of the calamus scriptorius. Each brain was then weighed to a tenth of a milligram in a closed weighing bottle and placed on absorbent cotton in a definite amount of 4 per cent formaldehyde. The glass stoppered bottles in which the brains were kept were of uniform size, and they were inclosed in black covered cases to exclude light as, according to Fish ('95), this precaution will prevent the decomposition of the solution and the subsequent formation of paraformaldehyde. The brains were weighed at definite times which varied somewhat in different series of experiments. On removal from the solution the brain was placed for a moment on filter paper to remove the superfluous liquid, it was then weighed as quickly as possible in a closed weighing bottle and returned to the solution. After a final weighing at the end of a stated period, the brains were dried for one week in a water bath which had a temperature of about 95°C. They were then cooled in a desiccator and reweighed in order to determine the effects of the solution on the percentage of solids in the brains. In some few cases, after the final weighing, brains were transferred into alcohol, imbedded by the celloidin-paraffine method of Bödecker ('08), and then sectioned and stained with thionin in order to ascertain the histological effects that had been produced.

THE WEIGHT CHANGES PRODUCED BY FORMALDEHYDE IN BRAINS
OF ALBINO RATS OF KNOWN AGES

Series 1. This was a preliminary set of experiments made to discover the general effects of a 4 per cent solution of formaldehyde on the brains of rats of different ages. In these experiments brains were taken from animals of the following ages: new-born, 10, 20, 40, 50, 70 days and adults, approximately 200 days old. Three rats of each age were used, animals of a given age being taken from the same litter except in the case of adult rats which were of unknown parentage and therefore may or may not have belonged to the same litter. As it was not possible to obtain all the rats wanted for the experiments at one time, the initial weighing of the first lot of brains was made early in October, 1910, while the final lot of material was not obtained until February, 1911. Each brain was put into 40 cc. of a 4 per cent solution of formaldehyde that was neutralized with NaCO_3 . The solution of formaldehyde used was, in some cases, one that had been made up for some weeks; in other cases a fresh solution was made as wanted, either from formalin that had been in the laboratory for some time or from a newly purchased supply. The age of the solution or the condition of the formalin used in making the solution were factors that were not thought to be of importance and therefore no attention was paid to them.

The different lots of brains were weighed at irregular intervals during the first week they were in the solution, then every seven days for nine weeks. At the end of this time each brain was transferred into a fresh solution, made at the time it was wanted for use. The bottles containing the brains were then sealed with paraffine and kept for two months at laboratory temperature. After eighteen weeks the brains received their final weighing and they were then dried to obtain the percentage of solids. In each set of brains of the same age the individual weighings were very uniform, as they showed a difference of only 2 or 3 per cent in the majority of cases. These differences can undoubtedly be ascribed to the fact that animals taken from the same litter often vary considerably in size even when they are of the same

sex, and with this difference in size is found a corresponding difference in brain weight since, as shown by Donaldson ('09), the size of the brain is correlated with the size of the animal, not with its age.

The times when the various weighings were made in this series of experiments, together with the average percentage weight increase for each group of three brains are given in table 1.

TABLE 1

Percentage weight increase in rats' brains kept for eighteen weeks in a stock solution of 4 per cent formaldehyde neutralized with NaCO₃ (averages for three brains at each age)

TIME SOLUTION ACTED	AGE OF RATS						
	New-born	10 days	20 days	40 days	50 days	70 days	200 days
1 day.....	21.6	30.0	28.5		25.8		
2 days.....	24.3		29.6				
3 days.....	26.7						
4 days.....	27.0 ¹				26.7		
5 days.....		31.5					
7 days.....	23.4	33.1 ¹	29.9 ¹	53.5 ¹	27.8 ¹	40.9	35.8
8 days.....		32.4	28.9		27.0		
9 days.....			28.3		25.4		
2 weeks.....	21.7	29.0	28.9	51.9	25.3	41.7	40.8 ¹
3 weeks.....	20.9	27.2	28.4	48.0	25.2	42.9	40.7
4 weeks.....	18.9	30.1	28.0	47.8	24.9	44.2	39.6
5 weeks.....	17.4	27.7	28.7	48.2	25.1	44.4	38.5
6 weeks.....	15.4	29.4	27.9	46.7	24.3	44.9	38.0
7 weeks.....	16.1	29.9	28.0	46.1	25.1	45.4 ¹	39.8
8 weeks.....	15.2	28.7	27.7	47.0	24.9	44.8	39.8
9 weeks.....	14.5	28.3	27.0	47.0	25.0	44.1	40.6
10 weeks.....	15.1	27.1	27.6	46.4	25.4	43.8	40.3
18 weeks.....	12.7	24.1	24.7	40.0	21.6	37.6	33.5
Average percentage gain.....	19.4	29.2	28.2	47.5	25.3	43.2	38.9

¹ Maximum weight increase.

The results of this series of experiments show that a 4 per cent solution of formaldehyde causes a pronounced swelling in the brains of rats of all ages. The maximum weight increase is reached, in most cases, during the first week, and there is then

a gradual decrease in weight until, after the brains have been in the solution for eighteen weeks, the percentage weight increase is reduced from 5 to 15 per cent below the maximum. In general, as shown in table 1, the amount of swelling seems to increase with the age of the rat up to the forty-day period, then it decreases slowly. Under the conditions of the experiments, therefore, the amount of swelling is not directly proportional either to the age of the animal or to the size of the brain.

Chart 1 shows the graph for the final percentage weight increase in the various sets of brains at the end of eighteen weeks.

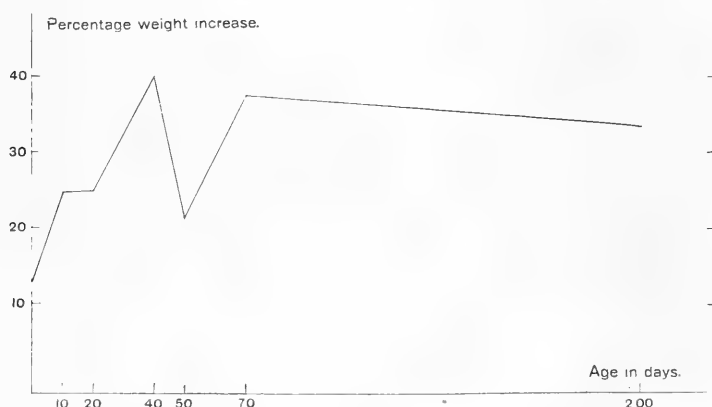


Chart 1 Showing the final percentage weight increase in a series of rats' brains kept for eighteen weeks in stock solutions of 4 per cent formaldehyde.

Starting relatively low the graph reaches its highest point with the forty-day group. Then comes a decided drop at fifty days, followed by a sharp rise at seventy days and a slight falling at the adult stage. As all the brains were kept in the same amount of solution under similar conditions of light and of temperature, the fall in the graph at the fifty-day period seems explicable only on the assumption that the stock solution of formaldehyde used in this instance had undergone some chemical change which had lessened its swelling action on the brain tissue.

As the stock solutions of 4 per cent formaldehyde used in this series of experiments had been kept in the laboratory for vary-

ing lengths of time, it seemed possible that the age of the solution might be a factor that would appreciably affect its swelling action on brain tissue. To test this assumption the following series of experiments was made.

Series 2. In this series, and in all others that were made subsequently, brains from rats of the following ages were used: new-born, 10, 20, 40, 50, 70, 100 and approximately 200 days old. Six rats of each age were used, the animals being taken from the same litter in order to avoid any possible variation in brain structure that might be characteristic of different litters. Three brains of each age were placed in a neutralized solution of 4 per cent formaldehyde that had been standing for five months; the remaining three brains of the same age were put into a neutralized solution that was made when the experiments were started. As all the animals were killed within a period of two weeks, the second solution was comparatively fresh when used on the final set of brains.

Each brain was put into 40 cc. of the solution and kept at laboratory temperature. The weighings were made on the first, third, and seventh days of preservation, also at the end of the second, third and tenth weeks. The specimens were then heat dried for one week and again weighed in order that the percentage of solids might be obtained.

Table 2 gives the data for the brains kept in the 'old' solution.

The data obtained in the experiments in which the brains were kept in the freshly made solution are given in table 3.

The results of these experiments are much more uniform than those obtained in the first series. The maximum amount of swelling was reached in all brains by the third day, and then there was a very gradual decline in weight until, at the end of ten weeks, the percentage weight increase was from 1 to 12 per cent below the maximum; in both sets of experiments the greatest loss in weight took place in the brains of new-born rats.

If we compare the data in table 2 with that in table 3, it is seen that the age of the solution used has a very marked effect on the amount of swelling in the brains at all ages. The old

TABLE 2

Percentage weight increase in rats' brains, each kept for ten weeks in 40 cc. of a neutralized solution of 4 per cent formaldehyde made five months before the experiments began (averages for three brains at each age)

TIME SOLUTION ACTED	AGE OF RATS							
	New-born	10 days	20 days	40 days	50 days	70 days	100 days	200 days
1 day.....	29.7 ¹	28.8	25.0	25.2	26.9 ¹	24.5	28.3 ¹	15.3
3 days.....	28.0	35.0 ¹	28.3 ¹	26.3 ¹	26.8	27.3 ¹	26.8	21.0 ¹
7 days.....	27.3	33.0	27.3	25.0	25.1	25.1	25.7	18.6
2 weeks.....	23.9	31.9	27.3	24.5	25.1	25.3	26.3	18.9
3 weeks.....	23.4	31.4	28.3	24.9	25.5	24.4	25.3	19.3
4 weeks.....	22.5	30.5	26.7	24.5	24.8	25.6	26.2	19.4
10 weeks.....	17.6	27.9	26.9	24.7	25.2	25.6	25.0	19.2
Average percentage gain...	24.6	31.2	27.1	25.0	25.6	25.4	26.2	18.8

¹ Maximum weight increase.

TABLE 3

Percentage weight increase in rats' brains, each kept for ten weeks in 40 cc. of a neutralized solution of 4 per cent formaldehyde made at the time the experiments began (averages for three brains at each age)

TIME SOLUTION ACTED	AGE OF RATS							
	New-born	10 days	20 days	40 days	50 days	70 days	100 days	200 days
1 day.....	44.4 ¹	58.2	39.5	37.9 ¹	39.3 ¹	34.4	45.6 ¹	32.4
3 days.....	42.0	64.6 ¹	41.5 ¹	37.6	38.5	38.6 ¹	43.1	34.7 ¹
7 days.....	41.5	62.1	40.1	36.4	35.6	34.1	41.1	30.9
2 weeks.....	38.0	62.9	39.7	35.9	36.1	34.9	41.0	30.8
3 weeks.....	37.7	63.4	40.0	35.7	36.9	34.3	40.4	31.2
4 weeks.....	36.1	62.8	39.9	35.5	35.4	35.7	40.5	31.6
10 weeks.....	33.9	61.4	39.4	35.5	36.1	35.5	37.7	31.8
Average percentage gain.....	39.1	62.2	40.0	36.4	36.7	35.4	41.3	31.9

¹ Maximum weight increase.

solution causes less swelling in every case than the one that was comparatively fresh when used, the difference between the average percentage weight increase in the various sets of brains of like ages ranging from 10 to 31 per cent.

The final percentage weight increase in each set of brains at the end of ten weeks is shown graphically in chart 2. The general form of the graphs is much the same, yet the graph for the



Chart 2 Showing the final percentage weight increase in two series of rats' brains, each kept for ten weeks in a neutralized solution of 4 per cent formaldehyde. A, solution made when the experiments began; B, solution five months old when used.

brains kept in the old solution (B) is much lower at every point than that for the brains kept in the freshly made solution (A).

The marked difference in the effects of these two solutions is not due to the fact that the older solution had become acid by standing while the newer solution had remained neutral. Both solutions were tested with good litmus paper on December 15, 1911, and each was found to give a very slight alkaline reaction. Since solutions of formaldehyde are readily decomposed it is prob-

able that the older solution underwent some chemical change through standing. There was, however, no evidence of the formation of paraformaldehyde when the bottle containing the old solution was first opened; the liquid appeared perfectly clear and had the characteristic odor of formaldehyde. No analysis of the solution was made, so it is not possible to state just what changes had occurred in it.

The results of this series of experiments indicate unmistakably that the age of the animal is an important factor in determining the amount of swelling that the brain will undergo in 4 per cent formaldehyde. In general, the younger brains absorb a relatively greater amount of liquid than do the older ones. This result, which accords with the observations of Hrdlicka and of Donaldson ('94), can doubtless be ascribed, in part at least, to the fact that the brains of young rats contain normally a greater percentage of water than do the older ones (Donaldson '10). According to the observations of Watson ('03) medullation in the rat's brain does not begin until the eleventh day after birth. The non-medullation of the fibers and the deficiency in supporting tissue are also factors that in all probability tend to increase the amount of swelling in the young brains.

Series 3. These experiments were made to ascertain whether the amount of swelling in the brain placed in 4 per cent formaldehyde will be influenced by the neutralization or by the non-neutralization of the solution. Lee ('05) does not approve of the neutralization of formaldehyde solutions, and he states that the slightly acid reaction that is usually found "is, as a rule, an advantage;" Bayon ('05), on the other hand, believes that an acid solution of formaldehyde is injurious to nerve tissue, and he advises that all such solutions should be neutralized with NaCO_3 . In carrying out these experiments it was considered necessary that the age of the solution should not be a factor that could influence the results, and, therefore, a fresh solution was made from a common stock supply of formalin when each lot of animals was killed. The four rats of each age that were used were taken from the same litter. Two brains from each group were put into 40 cc. of a solution neutralized with NaCO_3 ;

the two other brains of the group were put into the same amount of solution which was not neutralized and which gave a faintly acid reaction when tested with litmus paper. As the second series of experiments had shown that there is comparatively little difference between the percentage weight increase in the various sets of brains at the end of four weeks and at the end of ten weeks, these experiments and all the later ones were terminated at the end of one month and the brains heat dried.

Table 4 shows the data obtained in the experiments in which the brains were subjected to the action of a neutralized solution.

TABLE 4

Percentage weight increase in rats' brains, each kept for four weeks in 40 cc. of a neutralized solution of 4 per cent formaldehyde made fresh for each lot of animals killed (averages for two brains at each age)

TIME SOLUTION ACTED	AGE OF RATS							
	New-born	10 days	20 days	40 days	50 days	70 days	100 days	200 days
1 day.....	60.4	54.7	45.8	47.6 ¹	50.4 ¹	44.9	44.2 ¹	36.1
3 days.....	65.8 ¹	58.5 ¹	52.9 ¹	47.4	47.7	48.8 ¹	42.7	40.1 ¹
7 days.....	65.4	58.5	48.3	45.6	45.1	44.2	38.3	36.2
2 weeks.....	65.1	58.4	48.9	45.3	44.8	43.2	38.6	33.0
3 weeks.....	64.8	58.2	48.9	44.7	45.2	43.9	38.8	34.7
4 weeks.....	61.7	57.8	50.4	45.1	45.4	44.9	39.3	34.9
Average percentage gain.....	63.4	57.7	49.2	45.9	46.4	44.8	40.3	35.8

¹ Maximum weight increase.

The data given in this table indicate that, under the conditions of these experiments, the amount of swelling which the brains undergo diminishes as the age of the animal increases. Brains of new-born rats swell enormously, the average percentage increase in three days, when the maximum is reached, being 65.8 per cent of the original brain weight, and there is a falling off of only 4 per cent at the end of four weeks. Brains of ten-day-old animals gain, on an average, only about 6 per cent less

than those of the new-born: those belonging to the 20, 40, 50 and 70-day groups swell nearly the same amount, showing a maximum weight increase of from 49 to 53 per cent. In older animals the maximum increase in weight is somewhat less, amounting to 44 per cent in the case of 100-day animals and 40 per cent in the adults. The weight changes for each set of brains during the four weeks the experiments were continued are shown in the series of graphs in chart 3.

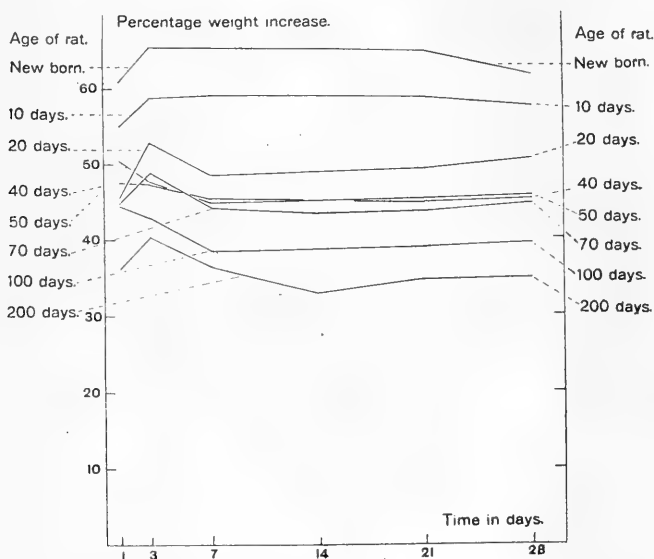


Chart 3 Showing the weight changes in brains of rats of various ages, each kept for four weeks in 40 cc. of a 4 per cent solution of formaldehyde neutralized with NaCO_3 .

As presumably all the solutions used in this set of experiments had the same chemical composition, the variations in the amount of swelling in the different groups of brains can doubtless be attributed to a difference in the chemical composition of the brains at different ages. The size of the brain, as will be shown later, is not a factor that influences the amount of swelling to any considerable extent.

The data obtained when brains were kept in a non-neutralized solution of 4 per cent formaldehyde for four weeks are given in table 5.

TABLE 5

Percentage weight increase in rats' brains, each kept for four weeks in 40 cc. of a non-neutralized solution of 4 per cent formaldehyde made fresh for each lot of animals killed (averages for two brains at each age)

TIME SOLUTION ACTED	AGE OF RATS							
	New-born	10 days	20 days	40 days	50 days	70 days	100 days	200 days
1 day.....	34.5 ¹	37.3	36.7	39.7 ¹	44.2 ¹	39.5	41.1 ¹	32.2
3 days.....	18.6	45.1 ¹	45.4 ¹	39.1	42.8	42.3 ¹	39.4	35.4 ¹
7 days.....	9.9	37.8	38.2	35.6	38.1	34.3	33.8	30.2
2 weeks.....	3.5	30.4	34.6	31.5	32.6	31.5	29.0	26.7
3 weeks.....	0.4	25.9	30.7	28.3	30.6	29.5	27.4	24.5
4 weeks.....	-1.5	23.5	27.9	26.6	27.8	27.3	24.3	24.5
Average percentage gain.....	13.1	33.3	35.6	33.5	36.0	34.1	32.5	28.9

¹ Maximum weight increase.

As shown in the table, the effects of an acid solution of formaldehyde on the brain of a new-born rat is most remarkable. The maximum weight increase amounts to only 34.5 per cent of the original brain weight, and it is attained at the end of the first day. There is then a rapid decrease in weight with each succeeding weighing until, at the end of four weeks, the brain actually weighs 1.5 per cent less than the original weight: this indicates that the solution has extracted some substance from the brain tissue. In both the brains of this age used the percentage weight changes were practically the same, as there was a difference between them of less than 2 per cent at any weighing.

Brains of ten-day-old rats do not show such remarkable weight changes as do those of new-born animals. In fact, an acid solution of 4 per cent formaldehyde causes nearly the same amount of swelling in brains of all ages from ten days to maturity, there being a difference of less than 10 per cent between the maximum weight increase in any two sets of brains, and a difference of

less than 5 per cent between the final weights. The very great difference between the effects of the solution on the brains of new-born animals and those on the brains of other ages is brought out very clearly in the graphs in chart 4 which show the percentage weight changes for each set of brains during the four weeks the experiments were continued.

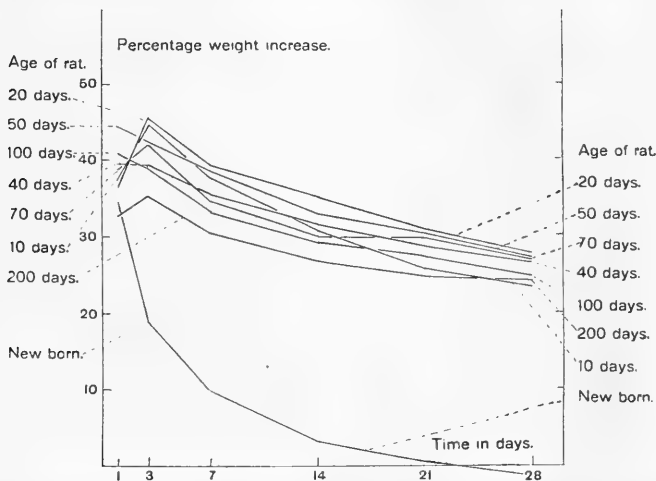


Chart 4 Showing the weight changes in brains of rats of various ages, each kept for 4 weeks in 40 cc. of a non-neutralized solution of 4 per cent formaldehyde.

This series of experiments shows that the neutralization or non-neutralization of a 4 per cent solution of formaldehyde has a marked effect on the amount of swelling of the brain tissue. The difference is shown in the graphs *A* and *B* of chart 5 which are plotted from the final percentage weight increase in the various sets of brains. As the weight of the brains of the new-born rats kept in the neutralized solution is some 62 per cent greater than that of the brains of the same age treated with the acid solution, the two graphs are very far apart at their beginning. At the ten-day period the difference between the graphs is reduced nearly one-half. They then approach gradually, and at the end are comparatively close together as the final weighings of the two sets of adult brains differed by only 10 per cent.

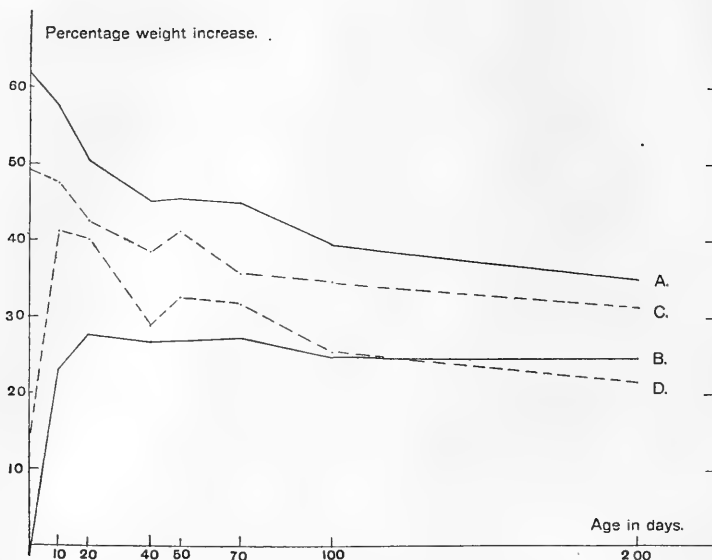


Chart 5 Showing the final percentage weight increase in series of rats' brains kept for four weeks in different quantities of neutralized and of non-neutralized solutions of 4 per cent formaldehyde. A, 40 cc. of a neutralized solution used; B, 40 cc. of a non-neutralized solution used; C, 20 cc. of a neutralized solution used; D, 20 cc. of a non-neutralized solution used.

Series 4. As it is known from the experiments of Donaldson ('94), Hrdlicka and others, that the amount of liquid in which brains are kept has a decided effect on the weight increase, the present series of experiments was made to test this point with brains of rats of various ages. The experiments were made exactly like those in Series 3, except that the amount of solution used was reduced from 40 to 20 cc. in every case. The data obtained in the experiments in which the brains were kept for four weeks in 20 cc. of a neutralized solution are given in table 6.

A comparison of the data given in this table with that in table 4 shows that the maximum, as well as the average, percentage weight increase is much lower when brains are treated with 20 cc. of solution than when double this amount of solution is used. This is the result one would expect if the amount of swelling diminishes as the strength of the solution is increased;

for the dilution of the solution by the tissue fluids is greater when a small amount of solution is used, and the effect is then the same as if the brains were kept in a weaker solution. According to the observations of Hrdlicka, the weight increase in brains treated with unneutralized solutions of formaldehyde is "larger with the weakest solutions and decreases as the proportion of formalin increases." It is evident, therefore, that in these experiments some factor, possibly the NaCO_3 used in neutralizing the solutions, has checked the swelling action of the weakened solu-

TABLE 6

Percentage weight increase in rats' brains, each kept for four weeks in 20 cc. of a neutralized solution of 4 per cent formaldehyde made fresh for each lot of animals killed (averages for two brains at each age)

TIME SOLUTION ACTED	AGE OF RATS							
	New-born	10 days	20 days	40 days	50 days	70 days	100 days	200 days
1 day.....	54.6 ¹	50.5	42.7	41.2 ¹	46.6 ¹	39.9	40.1 ¹	32.9
3 days.....	53.3	51.8 ¹	46.4 ¹	40.8	44.3	42.4 ¹	38.5	35.6 ¹
7 days.....	50.1	49.9	43.3	38.7	41.4	37.5	35.3	32.3
2 weeks.....	50.4	49.2	39.3	38.1	40.8	35.7	34.8	31.3
3 weeks.....	47.9	49.5	40.6	38.7	40.5	36.0	34.3	31.0
4 weeks.....	49.1	47.6	42.5	38.2	41.0	35.8	34.2	31.2
Average percentage gain.....	50.9	49.7	42.5	39.3	42.4	37.9	36.2	32.4

¹ Maximum weight increase.

tion. This seems probable from the results obtained in the second set of experiments in this series which show that a weak acid solution of formaldehyde causes a greater amount of swelling than does a stronger one.

The final weight changes for the various groups of brains are plotted in the graphs in chart 6.

Table 7 gives the data obtained in the experiments in which brains of different ages remained for four weeks in 20 cc. of a non-neutralized solution of 4 per cent formaldehyde.

In this instance brains of new-born rats do not show such striking weight changes as are shown in table 5. The initial rise

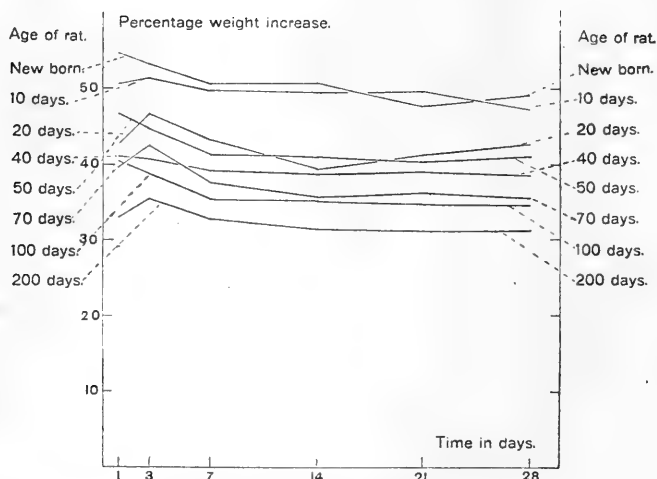


Chart 6 Showing the weight changes in brains of rats of various ages, each kept for four weeks in 20 cc. of a neutralized solution of 4 per cent formaldehyde.

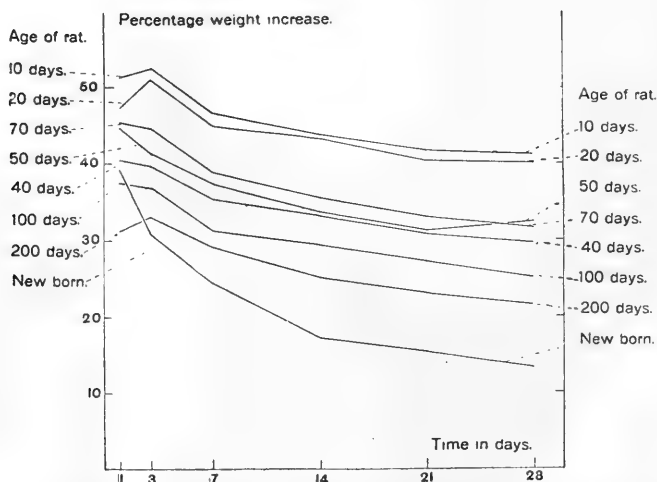


Chart 7 Showing the weight changes in brains of rats of various ages, each kept for four weeks in 20 cc. of a non-neutralized solution of 4 per cent formaldehyde.

TABLE 7

Percentage weight increase in rats' brains, each kept for four weeks in 20 cc. of a non-neutralized solution of 4 per cent formaldehyde made fresh for each lot of animals killed (averages for two brains at each age)

TIME SOLUTION ACTED	AGE OF RATS							
	New-born	10 days	20 days	40 days	50 days	70 days	100 days	200 days
1 day.....	38.9 ¹	51.5	47.1	40.2 ¹	44.5 ¹	45.1 ¹	37.7 ¹	31.1
3 days.....	31.1	52.1 ¹	51.3 ¹	39.9	41.3	44.8	36.9	33.1 ¹
7 days.....	24.2	46.5	44.9	35.2	37.4	38.6	31.4	29.2
2 weeks.....	17.2	43.9	43.3	33.1	32.9	35.7	29.3	25.1
3 weeks.....	15.5	41.9	40.4	30.7	31.5	33.7	27.1	23.2
4 weeks.....	13.4	41.1	40.0	28.9	32.2	31.9	25.3	21.9
Average percentage gain.....	23.4	46.1	44.6	34.7	36.6	38.3	31.3	27.3

¹Maximum weight increase.

of 38.9 per cent, found at the end of the first day, is greater by 4 per cent than that found in the previous set of experiments, but the subsequent fall in weight is not so rapid and at the end of four weeks the brains still weigh an average of 13.4 per cent above the original weight and therefore do not appear to have lost any of their substance. In these experiments the greatest percentage gain in weight occurs in the brains of the ten-day and twenty-day-old animals, the average gain for the entire period over which the weighings extended being in each case more than 40 per cent of the fresh weight: the data for the brains of animals from 40 to 200 days old differ but slightly from the corresponding data in table 5. The weight changes in the groups of brains of different ages are plotted in chart 7.

The contrast between the results of this series of experiments and those of Series 3 is brought out sharply in chart 5. Graph *C* was plotted from the final weights of the brains kept in 20 cc. of a neutralized solution of 4 per cent formaldehyde; graph *D* was plotted from data obtained where 20 cc. of a non-neutralized solution was used. These graphs run, for the most part, between the graphs *A* and *B*, which were plotted from the final brain

weights given in tables 4 and 5. Where the solutions were neutralized the form of the graphs is practically the same whether 40 or 20 cc. of the solution was used, but graph *C* falls considerably below graph *A* at every point. Where the solutions were not neutralized the form of the graphs show more variation, but graph *D*, for most of its length, runs higher than graph *B*. Since the diluent action of the tissue fluids is undoubtedly greater when 20 instead of 40 cc. of solution is used, it is evident that a stronger neutralized solution of 4 per cent formaldehyde causes more swelling in brain tissue than does a weaker neutralized solution, whereas the reverse is the case where the solutions are not neutralized.

Series 5. Since the temperature at which a solution acts is known to have a marked effect on the rate at which the solution will be absorbed, a final series of experiments was made to ascertain how different temperatures would affect the swelling of rats' brains in 4 per cent formaldehyde. In all of these experiments each brain was put into 40 cc. of a solution that was freshly made and neutralized when wanted for use. The bottles containing one set of brains were kept in a water bath at a constant temperature of 36°C. for four weeks: the corresponding set of brains remained at a temperature of 8 to 11°C. for the same length of time.

The data for the brains kept at the higher temperature are given in table 8.

In these experiments, as shown in the table, the maximum weight increase was reached in every case at the end of the first day. The decrease in weight at three days was practically the same for the brains of all ages, amounting to about 8 per cent. Subsequent weight changes were comparatively slight and, except in the very young brains, the final weighings differed but little from those noted for the third day. In this instance, also, there is a direct relation between the age of the animals and the percentage increase in brain weight, but the average percentage gain for the entire set of brains is considerably less than that found in the experiments in which the brains were kept in a neutralized solution at room temperature (table 4). These results accord

TABLE 8

Percentage weight increase in rats' brains, each left for four weeks in 40 cc. of a neutralized solution of 4 per cent formaldehyde kept at a constant temperature of 36°C. (averages for two brains at each age)

TIME SOLUTION ACTED	AGE OF RATS							
	New-born	10 days	20 days	40 days	50 days	70 days	100 days	200 days
1 day.....	61.5 ¹	59.5 ¹	50.9 ¹	51.8 ¹	45.5 ¹	44.3 ¹	39.3 ¹	38.8 ¹
3 days.....	53.6	51.6	42.9	43.9	37.8	36.6	31.7	32.7
7 days.....	48.3	49.6	43.6	43.2	37.4	36.5	31.1	30.4
2 weeks.....	50.4	48.4	43.7	44.7	38.1	36.9	32.8	31.6
3 weeks.....	42.3	47.8	44.2	44.8	38.6	38.8	32.4	31.5
4 weeks.....	40.6	48.3	45.0	44.8	38.2	39.3	33.3	32.6
Average percentage gain.....	49.1	50.9	45.0	45.5	39.3	38.7	33.4	32.9

¹ Maximum weight increase.

with Donaldson's ('94) observations that brains of sheep kept in a 2 per cent solution of bichromate of potassium at a temperature of 38°C. attain their maximum weight at a very early period and gain relatively less than when kept at a temperature of 10 to 17°C. In the case of the rats' brains kept at a temperature of 36°C. the decrease in the amount of swelling can be attributed, in part at least, to the fact that this temperature partially decomposes the solution of formaldehyde and liberates a considerable amount of formaldehyde gas. This of course weakens the solution, and a weaker solution of formaldehyde that has been neutralized with NaCO₃ does not cause as much swelling in rats' brains as does a stronger one, as was shown in the experiments in Series 4.

Graphs for the weight changes in the various sets of brains kept at a temperature of 36°C. are shown in chart 8. All of these graphs, it may be noted, are grouped in pairs according to the age of the animals. While a paired arrangement of some of the graphs is to be found in other charts (6, 7 and 9), in no case is the phenomenon as marked as in chart 8.

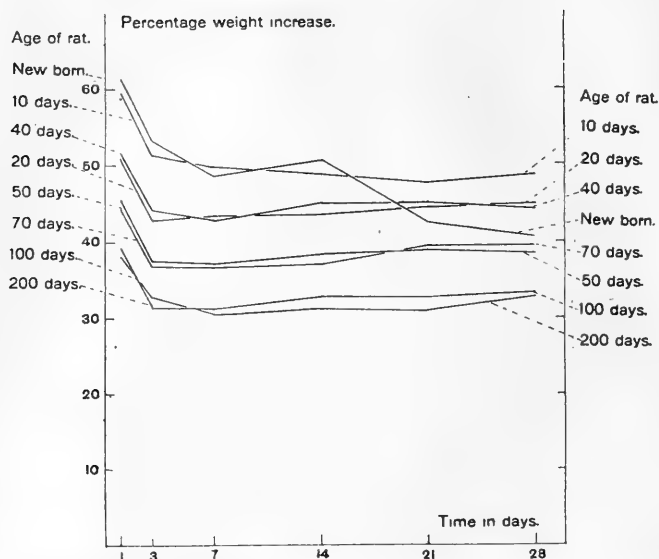


Chart 8 Showing the weight changes in brains of rats of various ages, each remaining for four weeks in 40 cc. of a neutralized solution of 4 per cent formaldehyde kept at a constant temperature of 36°C.

Table 9 gives the data for the weight changes in the brains kept in 4 per cent formaldehyde for four weeks at a temperature of 8 to 11°C.

As indicated in table 9, the maximum weight increase in all sets of brains was attained on the third day except in the case of the brains of the new-born rats where, as in most of the previous experiments, the maximum increase comes at the end of the first day. The subsequent loss in weight is very slight and it does not amount in any case to more than 8 per cent of the original brain weight. While the brains of young rats (birth to 40 days) show a relatively greater weight increase than the brains of older animals, there is not the very uniform decrease with advancing age that was noted in the previous set of experiments (table 4) where the brains were kept in 40 cc. of a neutralized solution at ordinary room temperature (about 20°C.), neither is the average increase for the various groups of brains as high.

TABLE 9

Percentage weight increase in rats' brains, each left for four weeks in 40 cc. of a neutralized solution of 4 per cent formaldehyde kept at a temperature of 8 to 11°C. (averages for two brains at each age)

TIME SOLUTION ACTED	AGE OF RATS							
	New-born	10 days	20 days	40 days	50 days	70 days	100 days	200 days
1 day.....	55.9 ¹	41.6	37.3	38.0	31.3	36.7	30.9	30.8
3 days.....	51.6	53.8 ¹	43.6 ¹	48.3 ¹	34.0 ¹	37.0 ¹	31.5 ¹	33.4 ¹
7 days.....	49.3	48.8	42.9	40.4	31.1	34.3	28.4	31.1
2 weeks.....	50.7	48.0	38.7	40.1	30.3	33.8	28.7	29.6
3 weeks.....	47.9	47.7	39.2	40.4	29.9	33.7	27.4	29.2
4 weeks.....	47.9	45.7	38.9	40.4	30.0	33.5	27.9	28.4
Average percentage gain.....	50.6	47.6	40.1	41.3	31.1	34.7	29.1	30.4

¹ Maximum weight increase.

This latter result is what might be expected, since a low temperature tends to lessen the amount of absorption of a liquid by brain tissue. This is the only set of experiments in which the average gain in the brains of adult rats is greater than that in the brains of 100-day-old animals. The increase, however, amounts to only about 1 per cent, so it is probably merely a chance variation.

Chart 9 shows the graphs plotted for the weight changes in the brains kept at a low temperature. There is a tendency here also to a paired arrangement of the graphs according to age, but it is not as pronounced as in chart 8.

The final percentage gain in weight for the two sets of brains used in this series of experiments is shown by graphs in chart 10. The form of the graphs is much the same, but the graph for the brains kept at relatively high temperature (*A*) runs somewhat higher than that for the brains kept at a low temperature (*B*). A difference of 25°C. in the temperature of the solutions in which the brains are kept has but comparatively little effect on the final weight increase at the end of four weeks

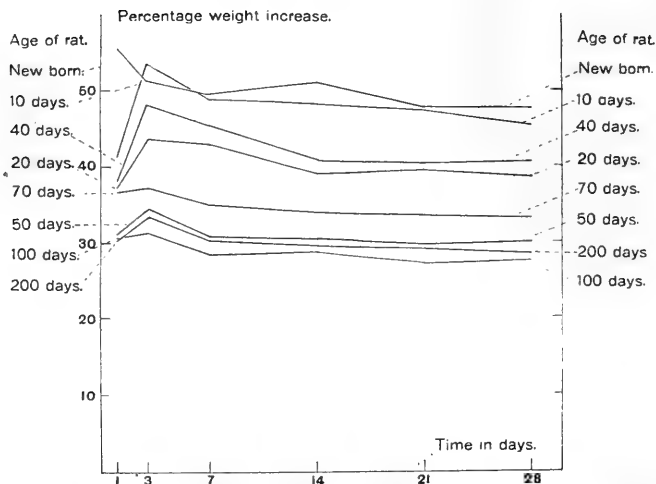


Chart 9 Showing the weight changes in brains of rats of various ages, each remaining for four weeks in 40 cc. of a neutralized solution of 4 per cent formaldehyde kept at a temperature of 8 to 11°C.

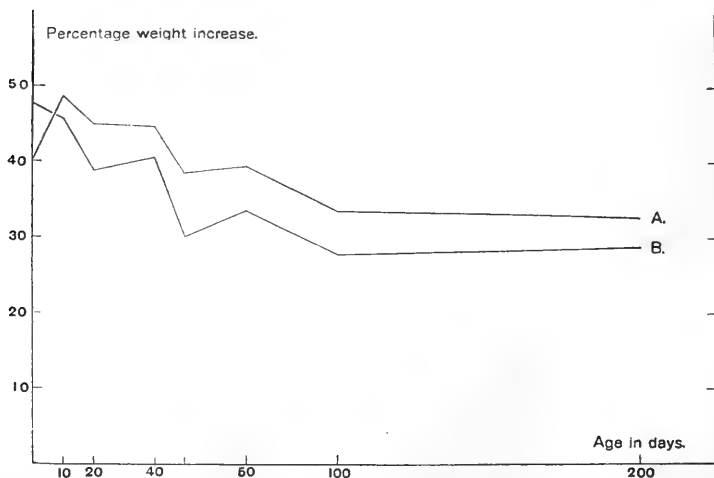


Chart 10 Showing the final percentage weight increase in two series of rats' brains kept at different temperatures during the four weeks they remained in a neutralized solution of 4 per cent formaldehyde. A, solutions kept at a temperature of 36°C.; B, solutions kept at a temperature of 8 to 11°C.

THE EFFECTS OF 4 PER CENT FORMALDEHYDE ON THE PERCENTAGE OF SOLIDS IN THE BRAIN OF THE ALBINO RAT

All the brains used in the above series of experiments, with the few exceptions that will be noted later, were heat dried for one week at the end of the weighing period to obtain the amount of dry substance. The percentage of solids left in the brains after treatment with the formaldehyde solutions was then calculated from the weight of the brains when fresh. A summary of the results obtained for the brains of each age is given in table 10. This table shows also the normal percentage of solids in the brains of rats of various ages as deduced from data given by Donaldson ('10) in his paper, "On the percentage of water in the brain and in the spinal cord of the albino rat."

As shown in this table, the normal percentage of solids in the brain of the rat, as deduced from Donaldson's data, varies directly with the age of the animal, being 12.2 per cent of the total brain weight in new-born rats and 21.6 per cent in the brains of adults. The computations made for the brains used in these various series of experiments show that in every case the percentage of solids is less than that normally found in fresh brains at the age observed, and that it increases from birth to maturity in a regularly striking manner. It follows from this that formaldehyde solutions extract some of the solids from the brain tissues. Brains of new-born rats suffer the greatest loss of solids; brains of adults are least affected.

The amount of solids lost from the brain of the rat through the action of a formaldehyde solution is not directly correlated either with the age of the animal or with the size of the brain. Brains of very young animals (birth to 10 days) lose about 30 per cent of their solids after treatment with a solution of formaldehyde. The average loss from the brains of older animals falls from 7.4 per cent in the 20-day-old brains to 1.5 per cent in the brains of animals 40 days of age. In brains of 50-day-old rats there is an increase in the extraction of solids by formaldehyde amounting to 6.2 per cent. The relative loss of solids from the brains of 40- and of 50-day-old animals, as shown in table 10, is not due to exceptional records for certain rats at these ages,

but it is found in practically all the individual records for all series of experiments. An examination of the data given in tables 1 to 9 shows that in six cases the average weight increase in the brains of the 50-day-old rats is greater than that in the brains of 40-day animals. These facts seem to indicate that some chemical change takes place in the brain at 50 days of age that has not been brought out by any analysis so far made.

TABLE 10

The percentage of solids in brains of rats of various ages kept from four to eighteen weeks in solutions of 4 per cent formaldehyde (computations made from original brain weights)

EXPERIMENTS	AGE OF RATS							
	New-born	10 days	20 days	40 days	50 days	70 days	100 days	200 days
Brains kept 18 wks. in neutralized stock solutions....	8.1	10.3	14.7	18.4	19.4	19.5		20.9
Brains kept 10 wks. in sol. 5 mos. old.....	8.1	10.1	16.5	19.4	19.4	20.5	19.7	20.5
Brains kept 10 wks. in freshly made sol.....	7.8	10.3	16.0	19.2	19.5	20.1	20.1	21.6
Brains kept 4 wks. in 40 cc. neutral sol.....	8.2	10.1	16.4	19.3	19.6	19.6	20.9	21.8
Brains kept 4 wks. in 40 cc. acid sol.....	9.6	10.9	16.7	19.3	19.1	20.7	20.1	21.1
Brains kept 4 wks. in 20 cc. neutral sol.....	9.2	9.8	16.2	19.7	20.5	19.9	20.2	21.5
Brains kept 4 wks. in 20 cc. acid sol.....	10.5	10.9	16.3	19.0	20.0	20.1	20.8	21.6
Brains kept 4 wks. in neutral sol. at temp. 26°C.....	9.7	9.8	15.1	18.7	19.4	19.8	20.1	20.1
Brains kept 4 wks. in neutral sol. at temp. 8 to 11°C.....	8.3	10.6	16.3	19.2	19.0	20.1	20.1	21.7
Averages for above series....	8.6	10.6	16.3	19.2	19.6	20.1	20.3	21.2
Normal percentage of solids in rats' brains (Donaldson)	12.2	14.6	17.5	19.5	20.9	21.1	21.3	21.6
Percentage loss of solids as result of action of formaldehyde.....	29.5	29.4	7.4	1.5	6.2	4.7	4.7	1.8

Brains of 70- and of 100-day-old rats suffer about the same relative loss in substance when kept in a 4 per cent solution of formaldehyde, amounting to about 5 per cent. The extraction from adult brains is about 2 per cent, showing that formaldehyde has little solvent action on the brains at this stage.

THE EFFECTS OF A 4 PER CENT SOLUTION OF FORMALDEHYDE ON THE BRAINS OF RATS INFECTED WITH PNEUMONIA

In the course of a study of the effects of pneumonia on the brain of the rat (King '11) a small series of experiments was made to ascertain whether the brains of animals suffering with this disease would react as do the brains of healthy rats when placed in a 4 per cent solution of formaldehyde. Five adult rats which were in advanced stages of pneumonia were killed with ether, and the brains removed at once and weighed. Each brain was placed in 40 cc. of a stock solution of 4 per cent formaldehyde which had been neutralized with NaCO_3 . The brains were kept in the solutions for one week, then weighed and the percentage weight increase calculated. For control purposes brains of five adult rats that were not suffering from any disease as far as could be determined were treated in a similar manner. The exact age of the rats used in these experiments was not known in any case, but all the rats were from five to eight months old. The age of the animal, therefore, was not a factor that could have had any appreciable influence on the results. Table 11 shows the fresh brain weights of the control and of the infected animals together with the percentage weight increase at the end of one week.

As shown in table 11, the average fresh brain weight as well as the percentage weight increase after treatment with 4 per cent formaldehyde are practically the same for both groups of brains used in this series of experiments. It would seem, therefore, as if pneumonia, even in its advanced stages, does not produce any changes in the brain tissues that affect the amount of swelling of the brains when kept in a solution of formaldehyde. An examination of the individual records, however, point to a dif-

ferent conclusion. The lowest brain weight in the infected group is 1.5915 gms. This weight is exceptionally low for the brain of an adult rat and it is about that found in a healthy rat of 50 days of age, as may be seen from table 12. The rat in question, therefore, was probably a 'runt' and cannot properly be classed with animals of normal size.

TABLE 11

	CONTROL ANIMALS		INFECTED ANIMALS	
	Fresh brain weight in grams	Percentage weight increase after one week in 4 per cent formaldehyde	Fresh brain weight in grams	Percentage weight increase after one week in 4 per cent formaldehyde
	1.7335	37.85	1.5915	24.53
	1.7501	36.47	1.7612	36.34
	1.7671	35.08	1.7917	40.44
	1.7867	39.34	1.8072	43.81
	1.8471	46.60	1.8889	48.08
Average of above records....	1.7769	39.05	1.7681	38.64
Average of last four records.	1.7887	39.38	1.8122	42.18

TABLE 12

Normal brain weights of albino rats of various ages and derived from data given by Donaldson ('08, '09)

AGE	MALES		FEMALES	
	Body weight in grams	Brain weight in grams	Body Weight in grams	Brain weight in grams
New-born.....	5.4	0.280	5.2	0.241
10 days.....	12.2	0.827	12.1	0.852
20 days.....	19.8	1.154	21.6	1.179
40 days.....	42.2	1.432	43.7	1.422
50 days.....	58.7	1.531	59.4	1.513
70 days.....	106.6	1.699	99.8	1.656
100 days.....	165.4	1.817	150.4	1.765
200 days.....	246.3	1.920	197.6	1.832

If we omit from table 11 the lowest record, both for the control and for the infected group, the remaining brain weights come fairly within the limits of probable normal variation in the brain weight of adult rats, as indicated in table 12. With this modification in table 11, the brains of the infected animals are, on the whole, slightly heavier than those of the controls, and the average percentage weight increase in these brains after treatment with 4 per cent formaldehyde is 2.80 per cent greater than that in the control group. Comparing the percentage weight increase in the brain of each of the infected animals with that of the control of the nearest brain weight, it is found that in every case the percentage weight increase in the brain of the infected animal is somewhat greater than that in the brain of the normal animal. It has been shown that the brain of an animal infected with pneumonia contains from 0.4 per cent to 0.5 per cent less water than the brain of healthy animals of about the same age (King '11). Pneumonia, therefore, not only decreases the percentage of water in the brain of the rat, but it also produces changes which cause the brain to undergo a slightly greater percentage weight increase when subjected to the action of a 4 per cent formaldehyde.

The greater percentage weight increase in the brains of the infected group of animals is not due to the fact that the brains of these animals were larger than those of the controls. In animals of the same age there is often a considerable variation in the brain weight, due to the fact that large animals have heavier brains than do smaller ones. An examination of the records of the 69 sets of brains of various ages used in these different series of experiments show that in 34 cases only does the larger brain undergo a greater percentage weight increase in 4 per cent formaldehyde. It is the age of the animal, not the size of the brain, that is a factor in determining the amount of swelling that the brain will undergo in a solution of formaldehyde.

THE HISTOLOGICAL EFFECTS OF FORMALDEHYDE ON THE BRAIN
OF THE ALBINO RAT

The histological changes produced in the cell structures of the brain of the albino rat by various formaldehyde solutions have been described in a previous paper (King '10). In general, as therein stated, such solutions "give a good fixation of the cell body, but they tend to produce a swelling of the nucleus which is usually accompanied by a poor preservation of the nuclear contents." In order to determine whether the conditions which so appreciably affect the amount of swelling of brain tissue in 4 per cent formaldehyde also produce histological changes in the cell structures, preparations were made of some of the brains of 100-day-old rats used in the experiments described above. After their final weighing at the end of a stated period, these brains were transferred into alcohol and imbedded and stained in the manner recommended in a previous paper (King '10). A careful examination was then made of the large cells in the cerebral cortex at the level of the optic chiasma.

In a brain that had been subjected to the action of 40 cc. of a neutralized solution of formaldehyde for four weeks the cell outlines were clearly defined, but somewhat irregular; the nuclei, however, were greatly distended and the nuclear contents were badly preserved. In many cases, also, the cytoplasm appeared vacuolated. In a brain kept in 40 cc. of a non-neutralized solution for four weeks, the nuclei appeared fully as much swollen as in the previous case, but the chromatin contents were far better preserved; the cell outlines stained more sharply and were somewhat more regular; and the cytoplasm was not vacuolated. Where the amount of solution used was reduced to 20 cc., the fixation of the cell structures, both in the brain that had been kept in a neutralized solution and in the one that had been subjected to the action of a slightly acid solution, the fixation of the cells was about the same as when double the amount of solution had been used. For histological preparations of brain structure, therefore, a neutralized solution of formaldehyde does not, apparently, have the advantage claimed by Bayon, for it greatly

increases the amount of swelling of the brain and has a correspondingly bad effect on the cell structures.

Keeping the formaldehyde solution at a temperature of 36°C. produces marked changes in the entire brain. By the third day the brains have a soft, gelatinous appearance which is not found in brains kept in a solution of formalin at room temperature or below. Preparations of these brains are practically useless for histological purposes, as the nuclei are greatly distorted and only faint traces of chromatin can be detected. A brain kept in a neutralized solution at a temperature of 8 to 11°C. for four weeks shows a fixation of the cell structures about like that obtained when the solution remains at laboratory temperature, but the cell outlines are sharper and the cell contents stain much better.

It is evident, from the results of these investigations, that conditions which affect the amount of swelling of brains in 4 per cent formaldehyde also affect the preservation of the cell structures in the brain tissue. If, therefore, it is considered necessary or advisable to preserve brains in formalin, the solution should not be neutralized and it should be used at relatively low temperature. This will insure a minimum amount of swelling and permit good staining. A prolonged immersion in the solution is unnecessary and decidedly injurious to the tissue cells. If the brain is ever to be used for histological purposes it should be transferred into alcohol as soon as it is fixed and hardened. Nerve tracts are apparently not adversely affected by a formaldehyde solution, and material so preserved can be stained by the Weigert method and used for investigations on the extent of medullation or of degeneration. A solution that swells brains from 30 to 60 per cent of their original weight in three days is obviously not an ideal cell fixative, and brain tissue preserved in formalin is therefore unfit for cytological work. It is more trouble, perhaps, to fix brains in Bouin's ('97) fluid or in the solution of Ohlmacher ('97), both of which give very excellent preparations of cell structures (King '11), but the superiority of these fixatives over a simple aqueous solution of formaldehyde cannot be questioned.

SUMMARY

1. A 4 per cent solution of formaldehyde causes a pronounced swelling in the brains of rats of all ages.

2. A solution of formaldehyde undergoes some chemical change on standing, since a solution five months old causes less swelling in the brain of the rat than does a freshly made solution.

3. A 4 per cent solution of formaldehyde neutralized with NaCO_3 produces a much greater amount of swelling in the brain of the rat than does a solution that has a faintly acid reaction.

4. A strong neutralized solution of formaldehyde causes a greater percentage weight increase in the rat's brain than does a weak neutralized solution. A reverse result is obtained when the solutions are not neutralized.

5. If rats' brains are subjected to the action of a solution of formaldehyde that is kept at a constant temperature of 36°C ., they undergo a greater amount of swelling than is produced when the solution is kept at a temperature of 8 to 11°C . The maximum weight increase in the brains is reached by the end of the first day in the former case, and not until the third day in the latter case.

6. When the conditions under which the solution acts are uniform, the maximum weight increase in rats' brains subjected to the action of a 4 per cent solution of formaldehyde is attained in all cases by the third day, and there is then a gradual decrease in weight. Brains of very young animals tend to reach the maximum earlier than do those of older animals.

7. The percentage weight increase in rats' brains as the result of the action of a 4 per cent formaldehyde solution tends to be greater in the brains of young animals than in those of adults.

8. In animals of the same age the larger brain does not show a greater percentage weight increase after treatment with a solution of formaldehyde than does the smaller one.

9. A 4 per cent solution of formaldehyde extracts solids from the brains of rats of all ages. This is shown by the fact that the percentage of solids in brains that have been subjected to the action of such a solution is always less than that found in the fresh brains of animals of the same age. Brains of very young rats lose much more of their solids than do brains of older animals.

10. Brains of animals infected with pneumonia show a slightly greater percentage weight increase when treated with a 4 per cent solution of formaldehyde than do the brains of healthy animals.

11. Even under the most favorable conditions an aqueous solution of formaldehyde is not a satisfactory fixative for the cell structures in brain tissues, as it causes a pronounced distention of the nuclei and gives a poor preservation of the nuclear contents.

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MÖLLGAARD'S RETICULUM

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SIX FIGURES

Since the appearance of Möllgaard's article: "Die Vitale Fixation des Zentralnervensystems," in the *Anatomische Hefte* (Bd. 43, July, 1911) much comment has been made upon his method and his conclusions. Considerable difference of opinion still seems to exist however regarding his results, so it has been thought profitable to do some further work in this direction. Prominent among Möllgaard's conclusions is the fact that he strongly doubts the existence of Nissl's bodies and neurofibrillae in the 'vitaly-fixed' nerve cell. Thus, one of the objects of the present paper will be to determine whether or not Nissl's bodies do exist in tissue so fixed, and a few observations incidentally made on neurofibrillae will be included. An endeavor has also been made to ascertain how and why Möllgaard's 'glia-network' in neural tissue is formed during the process of freezing.

This investigation was undertaken on suggestion of Dr. C. M. Jackson by whose criticism and advice the author has greatly profited and for which aid he here expresses his indebtedness.

REVIEW OF RECENT LITERATURE

Möllgaard ('11 a) emphasizes the fact that to exclude the greatest number of artefacts a tissue should, before its study, undergo as little preparation as possible. He remarks, that the very simplest of methods is demanded; and, that with simpler technique comes ease in making controls. If one would succeed, he says, in studying the finest structural relationships of the living cell, one must, firstly, be prepared to study a definite physio-

logical function of the cell. Secondly, since a physiological function is progressive, one must, in order to study this condition, be prepared to interrupt that function at any given point. He would interrupt the functional state and immediately preserve the tissue in that vital condition until such time as would be convenient to continue the technique. The physiological processes of the central nervous system proceed with great rapidity and post-mortem changes enter in like manner, so for a physiological-histological investigation he regards our general neurocytological technique as unsuitable.

Möllgaard's paramount aim is, therefore, the elimination of post-mortem changes. To attain this end he takes the tissue from the living animal and quickly drops it into a freezing mixture in which it is at once frozen. Since at the low temperature (ca. $-40^{\circ}\text{C}.$) of the freezing mixture practically all chemical reactions are almost completely interrupted, Möllgaard maintains that the tissue is preserved in the same vital condition in which it was when committed to the fluid. The next step rests upon the fact that the central nervous system has, at a temperature of about $-20^{\circ}\text{C}.$, a consistency like that of paraffin and may be cut into sections of 5 to 10μ in thickness without previous fixation or embedding. The sections as fast as made are consigned to a fixative of similar temperature which at once fixes them and so permanently preserves the sections in a 'vitality-fixed' condition.

More in detail, Möllgaard's technique is briefly as follows: From the living animal (rats, dogs, etc.) upon which with the aid of an anaesthetic, he has previously performed a craniotomy or a laminectomy, he excises a small piece of brain or cord tissue, and immediately drops the same into a freezing mixture, consisting of carbon tetrachloride, xylol, and absolute alcohol. The temperature of this mixture has been reduced to about $-40^{\circ}\text{C}.$ by the addition of carbon dioxide snow. The animal is then killed, generally by cutting its throat, and pieces of the brain or spinal cord taken and placed in the freezing mixture at various intervals after death for the purpose of later comparison. After a sojourn of varying length in the freezing mixture, the

pieces of tissue are fastened to the object carrier of the microtome. The 'inner vessel of the calorimeter' containing the fixing fluid, generally 96 per cent alcohol, which by the addition of carbon dioxide snow has been reduced to a temperature of -15° to -35°C. , according to the desired thickness of the subsequent sections, is now brought up about the tissue block. At the appropriate temperature sections are cut with the specially constructed microtome into the cold fixative. The sections are thus fixed at once and at a low temperature. The fixative with the contained sections is now allowed gradually to acquire room temperature, after which the sections are studied in the unstained condition, or stained with toluidin-blue or nile-blue base.

After a study of the sections, obtained and prepared according to the foregoing technique, Möllgaard concludes:

Firstly, concerning the Nissl's bodies:

1. "Nissl-Körner existiren nicht in vital fixierten Nervenzellen."

2. "Nissl-Körner sind Kunstprodukte, hervorgerufen während der Alkoholfixation."

He notes, however, that it is not the alcohol alone that produces the Nissl's bodies, for by his method of 'vital fixation' he excludes a factor which is present in the usual Nissl's method, namely, post-mortem change. Since he has observed that the first result of post-mortem change is the production of a meshwork¹ in the unstained protoplasm, and a later result that of an aggregated or conglomerated meshwork ('zusammengeballtes Maschenwerk') in the stained protoplasm, he holds that the Nissl's bodies are due to the simultaneous action of post-mortem change and alcohol fixation. He regards the post-mortem changes as acid in character, stating that: "Die Nissl-Körner sind darum als Kunstprodukte anzusehen, und zwar als solche, die durch eine Verbindung der sauren postmortalen Spaltung und der lang-samen Alkoholfixation entstanden sind."

¹ This meshwork, however, seems to be practically identical with the 'glia-network' (to be described later) which Möllgaard finds in his 'vitaly fixed' nerve cell, and which he considers normal. Regarding this and some other points his various statements seem vague and inconsistent.

Although he finds no Nissl's bodies, Möllgaard's observes in the 'vitaly-fixed' and stained nerve cell a network, which he interprets as a 'glia-network,' and whose richness depends on the time after death the preparation is made. In the cells of a piece of tissue taken from the living animal and 'vitaly-fixed' and stained he finds a network consisting at most of three to four coarse meshes, while in cells of preparations made ten to twelve minutes after death the network reaches its greatest development. In 'vitaly-fixed' tissue the cell protoplasm lying between the meshes of this network remains wholly unstained with toluidin-blue for some time after the tissue is taken, and only becomes stainable one and one-half to four hours after death, during which time the reaction of the brain substance has become increasingly acid. As the time after death increases the cellular protoplasm within the meshes of the networks of the cells becomes more and more stainable. But at about fifteen to twenty hours post-mortem the network itself, on the other hand, begins to disintegrate and forms peculiar aggregated masses. The significance of these masses will be mentioned later.

The technique may be varied as follows: A small piece of nerve tissue is taken seven minutes after death, is frozen, and placed in 96 per cent alcohol at room temperature and left for one and one-half to two hours. Then it is cut and fixed in alcohol at $-20^{\circ}\text{C}.$, and finally stained with toluidin-blue. With this treatment Möllgaard says that the network begins to shrink and disintegrate. If the tissue is left in the alcohol at room temperature for about twelve hours before being cut and stained he finds an additional structure present, namely, granulations. These granulations, in his own words: "gleichen vollständig den Nissl-Körnern." The networks of the cells in which the granulations are present, he finds, on close examination, to be composed of a series of closely placed granules. Hence Möllgaard believes that in tissue taken at various intervals after death the networks of the 'vitaly-fixed' nerve cells gradually degenerate into the peculiar masses and granulations mentioned above, which are apparently Nissl's bodies. To quote again, he says: "Wir haben gezeigt oder jedenfalls in höchsten Grade

wahrscheinlich gemacht, dass die Nissl-Körner von diesen Netzen gebildet werden." The origin and nature of the network discussed now becomes Möllgaard's principal question. For the purposes of the present paper, however, it is not necessary to follow him through all the details of his attempts to explain its presence. But it might be added that his final conclusion regarding its nature is that it is a 'glia-network,' which is both intercellular and intracellular.

Secondly, regarding the neurofibrillae, Möllgaard's conclusion is evident from the following quotations:

Ich habe nun gleichzeitig an vital alkoholfixierten Schnitten die allgemeinen Fibrillmethoden versucht, doch überall mit negativem Ergebnis. . . . Auf Grund dieser Versuche darf ich nicht wagen, die Existenz der Neurofibrillen zu verneinen. Dazu scheint mir, dass ich die Verhältnisse vorläufig zu wenig untersucht habe. Doch kommt mir unleugbar vor, dass die eben erwähnten negativen Ergebnisse recht stark gegen die Existenz der Neurofibrillen sprechen. Falls dagegen Neurofibrillen Kunstprodukte gleichwie Nissl-Körner sind, so würden diese vermutlich ganz dasselbe Schicksal wie die Nissl-Körner erleiden, nämlich dass sie erscheinen, wenn man langsam fixiert und die postmortalen Prozesse ausgeschlossen sind, d. h. wenn man das Gewebe vital fixiert.

It may be further noted that he remarks: "Die Zellen lassen sich überhaupt sehr schwierig impragnieren." He is of course speaking of cells 'vitaly-fixed.' In speaking of the neurofibrillae he also says that one cannot deny that the appearances which the 'vitaly-fixed' nerve cells present lie nearest to the reality; and so one can maintain with considerable certainty that what is not present in the 'vitaly-fixed' nerve cell is likewise not present in the living cell.

Retzius ('11) strongly criticises Möllgaard for adopting a method which in all its essentials is like that which he and Axel Key discarded thirty-seven years ago. He says that Möllgaard without doubt started out with the right object in view, namely, to secure a simple method for the study of the central nervous system, realizing that our present methods demand too much preparation before the tissue is ready for study. But of the methods for the study of the central nervous system, Retzius

holds the freezing method to be one of the poorest. Of this fact he says he convinced himself in the year 1874, when he carried out a large number of experiments in this connection, some of which may be briefly mentioned. Under the microscope he followed the very formation of the network which Möllgaard describes at such great length. He likewise examined the freezing process in all its phases for a large series of tissues and fluids (blood, glue, egg-albumen, starch-paste, etc.) and arrived at the certain conviction that this 'vital' method is very treacherous for scientific purposes and should be strongly condemned. From his studies Retzius concludes that the network observed in the frozen preparations is due to distortions and lacerations brought about by a system of spaces, passages, lacunae, clefts, and tubules filled with crystals of ice. He says that if to the frozen preparations a fixing fluid (alcohol, osmic acid, etc.) is applied, the system of clefts and lacunae, with the distortions and lacerations incurred, is preserved in all detail; but, if on the contrary, the preparations are permitted to thaw out without previous fixation, the water reappears anew in the entire mass, and only here and there a small cleft is left behind. From a consideration of the entire phenomenon it is evident to him that the water contained in the tissue or the fluid mass at the moment of the freezing passes out of the parenchyma and collects itself in the passages and lacunae which then appear in the frozen condition filled with crystals of ice. The water apparently collects where it meets with the least resistance. The foregoing results and others arrived at by Retzius together with Key were published by them in the Swedish language in 1874 and in the German language in 1882, so they should have been quite accessible to Möllgaard. Retzius in brief affirms that Möllgaard's 'glia-network' is an artefact due to the technique employed, but farther than this he does not criticise Möllgaard's ideas and results.

Möllgaard ('11 b) in replying to Retzius' criticism, says that he very much regrets that the articles by Retzius and Key so long ago escaped his notice, for on duplicating some of their experiments he is forced to acknowledge that his 'glia-network'

is wholly an artefact. He, however, entertains the hope that the method may still be utilized in studies dealing with physiological and pathological changes in nerve tissue.

From the foregoing it will be observed that it may still be considered questionable:

1. Whether the Nissl's bodies and neurofibrillae are present in the freshly fixed nerve cell.

2. Whether freezing the fresh neural tissue at a low temperature so changes the cytological structure that neither Nissl's bodies nor neurofibrillae can be demonstrated.

3. Whether the Nissl's bodies and neurofibrillae are due to post-mortem changes.

4. What the relation is of Möllgaard's reticulum to the Nissl's substance.

5. How and why Möllgaard's reticulum, or 'glia-network,' is formed during the freezing.

The observations made in the present study will afford at least a partial answer to these questions.

MATERIALS AND TECHNIQUE

With one exception, the tissue studied was taken from the spinal cord of the dog. The exception was that of a horse killed by the Department of Veterinary Medicine of the University of Missouri. In addition to neural tissue, several other tissues and different fluid masses were used, but as these were employed expressly for control observations they will be referred to only as occasion demands. The number of dogs used was twenty-four. They were obtained, as a rule, the day before they were to be killed, from the city pound or from individual owners. Such public source of course means that the dogs were of varied breed and description. Care however was taken that only healthy, well-nourished, active dogs were used. Likewise much care was exercised in avoiding exciting or in any way injuring the animal before it was killed. It was sought in general to use adult dogs of medium size and of an age that did not lie below one year

nor above five years. The age of all the animals used lay well within the limits noted but their weight varied between 10 to 19.7 kgm.

Regarding the method of killing, the technique should produce the least possible change in the nerve cell and at the same time permit of taking the tissue in a minimal length of time. This excludes the use of anesthetics, narcotics, and even hypnotics, as well as death by shooting, hanging, stabbing, or asphyxiating. The method finally decided upon was one of decapitation with a double-guillotine, thus removing a segment of the neck including the spinal cord. The apparatus used is composed of two large butcher's cleavers, heavily weighted, and so bolted together that they can be adjusted according to the length of the segment of cord desired. The necessary accessories to the method consist of a block sufficiently firm to receive the impact of the blow of the decapitating apparatus without jolting, and thus permitting of obtaining the segment desired at one blow; and a strongly made animal-board that can be closely and carefully adjusted to the foregoing block. The animal-board is provided with straps permitting of fastening the dog upon it quickly and conveniently. The dog's neck, by means of a strap and collar, is stretched out upon the block. For recording the time of decapitation and the subsequent taking of the tissue a stop-watch was used. The various details of construction and other minor aspects of the method, so briefly and simply stated above, as well as the several little difficulties confronted in its use are obvious and readily remedied and so need no lengthy description.

In taking the tissue it is of course essential that the assistants² be fully instructed as to the particular place they fill, and that everything is in absolute readiness. For the decapitation is practically instantaneous and thereafter the tissue is immediately at the disposal of the investigator. The segment of the animal's

² It is with a feeling of deep obligation that the author expresses his indebtedness to Messrs. M. D. Ott, H. L. Kearney, M. M. Miller, J. S. Homan, S. H. Snider, W. H. Taylor, T. K. Kruse, and Geo. Klinkerfuss, for the assistance so willingly and kindly granted.

neck obtained by the decapitation contains a portion of the spinal cord varying from the first to the fifth cervical segments according to the adjustment of the apparatus and as to the particular place on the neck where the blow happens to fall. This neck segment should of course be obtained as soon as possible after the blow is struck. Then, with or without depriving the segment of its adherent musculature, a long slender knife is passed around the spinal cord just within its dural sheath. Simultaneously, one of the assistants carefully grasps an end of the cord and drawing it from the canal quickly hands it to the investigator. The isolated cord segment is now cleft longitudinally in the region of the anterior horns. From the exposed gray substance (anterior horns) smears are made on glass slides which are dropped into Coplin jars containing the fixing fluids. When the foregoing was carefully and successfully carried out it was found that the smears could be placed in the fixing fluids twenty-five seconds after the moment of decapitation.

Fixation was carried out both with and without freezing; that is, in the one case the jar containing the fixing fluid was surrounded by a freezing mixture while in the other case it was not. In general, 96 per cent alcohol was used as a fixative for Nissl's bodies; and, when the smears were frozen, for the neurofibrillae also. For fixing the neurofibrillae of unfrozen smears the fluid (24 parts absolute alcohol, and 1 part ammonia) recommended by London ('05) was more frequently used. For comparison and checking of results on the Nissl's bodies, Ohlmacher's fluid and formol-corrosive were used in a few cases. For a like purpose 12 per cent formol was used as an additional fixative for the neurofibrillae in a few instances. The length of time the smear preparations were left in the fixatives varied in the case of the Nissl's bodies from one-half hour to three days, and for the neurofibrillae from five hours to four days.

Fixation with freezing, to be more explicit, was carried out as follows: A medium sized vessel (capacity approximately one gallon) containing 95 per cent alcohol was placed within a larger container. In the smaller vessel with the alcohol were placed small Coplin jars containing 96 per cent alcohol and ten or twelve

glass slides respectively. The larger container was now filled with ice and salt which was carefully packed about the smaller vessel. When the temperature of the alcohol and the fixative had been reduced to $-8^{\circ}\text{C}.$ or lower, carbon dioxide snow was added to the 95 per cent alcohol in the smaller of the containers until the temperature was further reduced to -20° to $-50^{\circ}\text{C}.$ At this time the dog was immediately killed and smears made on the cold slides upon which they froze instantly. Then they were quickly dropped into the cold fixative. The time consumed in making the preparations was judged from the instant of decapitation to the exact moment the fixative received the smear. The smears were left in the fixative for a time varying as above noted. When the time was prolonged the whole of course acquired room temperature in the meantime.

In those cases where preparations were made at various intervals after death the cord segment was in all cases kept under the ordinary conditions of the laboratory and at ordinary room temperature. The majority of the long post-mortem interval preparations, however, were made during the winter months so it may be said that the temperature of the laboratory ranged from 5° to $23^{\circ}\text{C}.$

The staining methods used are comparatively simple. For the staining of the Nissl's bodies the method outlined by Dolley ('11) was used. The method is in the main as follows: The smears fixed in 96 per cent alcohol are brought through a series of alcohols of decreasing strength to distilled water, then stained with warm (ca. $40^{\circ}\text{C}.$) erythrosin for three minutes, and well washed in water. They are then brought into a 1 per cent aqueous solution of toluidin-blue for five to eight minutes, again well washed in water, after which they are dipped in 95 per cent alcohol and differentiated, until the Nissl's bodies and nuclear structures are clearly defined, in a mixture of 96 per cent alcohol 9 parts, anilin oil 1 part. The differentiation is stopped by bringing the slide into absolute alcohol from which it is brought into xylol and mounted in Canada-balsam or damar.

To stain the neurofibrillae, London's method was followed. The method is essentially the following: After fixation, the smears

are brought into a 1.5 per cent aqueous solution of silver nitrate, kept at a temperature of about 37°C. for three to seven days, if unfrozen, and for one to three weeks if frozen, after which time they are treated with a solution of pyrogallie acid, 2 grams, formal 5 cc., and distilled water 100 cc., for twenty-four hours. The smears are then placed in a 1 per cent aqueous solution of gold chloride for five to ten minutes, brought into a 5 per cent aqueous solution of sodium hyposulphite for ten minutes, carried through distilled water and a series of alcohols of increasing strength to xylol and mounted in damar or Canada-balsam.

In the case of the neural tissue, the foregoing methods were used almost exclusively. Variations were made only occasionally and in fact so rarely that they need not be mentioned, excepting that some of the smears were stained for Nissl's bodies without any previous fixation, and that in staining for neurofibrillae a few control preparations were made according to Legendre's ('06) modification of Bielschowsky's method.

For control purposes too, smear preparations of hepatic and pancreatic tissues were subjected to the above technique, and the freezing of distilled water and egg-albumen was carefully studied under various experimental conditions.

It is thought unnecessary to describe the various magnifications employed in the study of the smears. An ordinary Leitz oil immersion lens was in all cases quite sufficient to determine the points in question.

OBSERVATIONS

The observations made may be conveniently assembled under the following headings:

Nissl's bodies

1. In unfrozen smears
2. In frozen smears
 - a. Fresh, frozen, unfixed, and unstained smears
 - b. Frozen, fixed, and stained smears considered as a whole
 - c. Nerve cells of smears frozen at -5° to -10°C .
 - d. Nerve cells of smears frozen at -10°C . and lower

Additional observations

NISSL'S BODIES

1. In unfrozen smears

The observations on Nissl's bodies in nerve cells of unfrozen tissue may be very briefly considered.

In smears not subjected to freezing, but fixed in alcohol at ordinary room temperature the Nissl's bodies are present, clearly and definitely defined in cells fixed twenty-five seconds after decapitation (fig. 2). They were also present at all intervals thereafter until they were finally lost in the complete post-mortem disintegration of the tissue. Similarly Nissl's bodies are present, distinctly defined, in the nerve cells of smears placed, without the usual fixation, in the toluidin-blue stain one and three-fourths minutes after death. Not only are the Nissl's bodies distinctly present in the foregoing preparations, but also their form and their distribution in the cell body and the dendrites are clearly evident. In some of the best smears, and with good magnification, it is even possible to see that the Nissl's bodies are composed of granules which lie embedded in a matrix, the 'gerinnselartige Masse' of Held ('95). In Nissl's bodies in the periphery of a well differentiated cell this matrix appears of slightly purplish hue while the Nissl's granules are a deep blue. These observations on the matrix are in agreement with those of Held ('95 and '97) and Becker ('06).

2. In frozen smears

In the case of the frozen tissue the picture is quite different from that of tissue that has not been frozen, and as an aid to its interpretation a few preliminary notes on the effect of freezing on some fluids and fluid-masses may first be mentioned.

Since such a great percentage of all tissues and fluid masses is water and because so much separates out during the process of freezing, it is of interest to note the appearance of a frozen drop of water or of the ice as it fills the interstices of the tissue. The appearance is one of large and small prismatic and spherical foam-cells (Quinke) filled with clear pure or nearly pure congealed water with here and there small air bubbles between their

adjacent walls. This appearance is observed of course only in freshly frozen preparations, not after their fixation, and is mentioned only to insure a more correct association between the appearances of the aqueous and less aqueous portions of the preparations.

The appearance of ice in animal tissues seems to have been quite neglected, but its appearance in plant tissues has been variously noted by Müller-Thurgau ('80 and '86), Fischer ('11), Wiegand ('11), and others. The details of ice formation however need not be entered into here; for such details, with physico-chemical explanation, are given at length by Quincke ('05).



Fig. 1 This figure is a reproduction of Molisch's ('97) figure 6, with a reduction of one-sixth. It represents the reticulum observed in a thin film of starch-paste, first frozen and then permitted to thaw out.

The appearance of frozen egg-albumen is also instructive. Depending on the conditions of the freezing, many variations occur, but in general the appearance is one of a variable network or reticulum the meshes of which are filled with ice. Disregarding the variations, the network is not unlike that figured by Molisch ('97) for frozen starch-paste with subsequent thawing (fig. 1). Small air-bubbles are usually present, and intermingled with the reticulum, if the temperature employed be low enough, many small clefts occur.

It should be remarked that Molisch ('97 and '11) has made extensive observations on the action of freezing on many substances, solutions, and emulsions, colloidal and otherwise. From

his studies Molisch concludes: Freezing causes a separation of water; this water at numerous points forms ice crystals which by molecular force continue to extract water from the substance in question and by their consequent enlargement push aside the substance, and in this way give rise to the network or reticulum observed. Several of his figures, one of which is reproduced here in figure 1, strikingly illustrate this point.

The observations on various gums and other colloids all agree, in their essentials, with those of Molisch: Ambronn ('91); on a number of inorganic and organic colloids, at temperature reductions of -10° , -70° , and -180°C. by Bobertag, Feist, and Fischer ('08); on starch-paste, various gums, and hemoglobin, at temperatures as low as -180°C. , by Fischer ('11); and, on gelatin by Liesegang ('11). Likewise, except for some details, the observations of the author are in accord with those of the investigators named.

The observations on frozen neural tissue may be considered under the following divisions: (a) fresh, frozen, unfixed, and unstained smears; (b) frozen, fixed, and stained smears considered as a whole; (c) nerve cells of smears frozen at -5° to -10°C. ; (d) nerve cells of smears frozen at -10°C. and lower.

a. Fresh, frozen, unfixed, and unstained smears. A smear of gray matter from the spinal cord of dog, frozen over the gas-escape of a carbon dioxide tank or by making the smear on a very cold slide, becomes of whitish opacity and at very low temperatures develops clefts not unlike those observed in ice and frozen egg-white, though in general it takes a much lower temperature to develop such clefts in neural tissue. The appearance presented under the microscope varies considerably according to the thickness of the smear, the rapidity of the freezing, the degree of temperature reduction, and undoubtedly too on the surface tension and other intrinsic forces of the individual neural elements. In general, however, it may be said that small foam-cells resembling those of aqueous ice, variously arranged, are as a rule to be observed around the margin of the smear; the smear itself appearing as a coarse network or reticulum with large and small, irregular or multiangular meshes. Within the meshes is

the more liquid frozen matrix or ice, while the network itself consists of the more solid portions of the tissue. In many places the largest of the nerve fibers found in gray matter appear to form initial strands upon which the network is formed, in other places however they extend into or through the lacuna-like spaces quite unaccompanied by other neural elements. Regarding the various kinds of cells (nerve cells, blood cells, and glia cells) nothing can be made out with certainty in these frozen and unstained preparations.

Molisch ('97) has shown that the reticulum produced by freezing in such colloids as gelatin and starch-paste is more or less permanent after thawing out, others such as gum tragacanth, gum arabic, and egg-albumen the reticulum disappears quite completely. The author's observations on the last named colloid confirm this statement and so too for the non-fixed neural tissue described above no noticeable trace of the effects of the previous freezing can be noted after thawing.

b. Frozen, fixed, and stained smears considered as a whole. Smears frozen, fixed and stained according to the directions given under the heading 'Material and technique' show, when frozen at a temperature of -5° to -10°C. , the first effects of freezing. Like the fresh, frozen, unfixed, and unstained smear, the preparation, when looked upon as a whole, appears to consist of a more or less coarse network or reticulum the interstices of which are of variable size and form. With greater temperature reductions (-10° to -40°C.) this network becomes more definite and distinct, and presents a number of special features which will be discussed under other headings. The network observed is stained in part blue (by the toluidin-blue) and in part pink (by the erythrosin). Coarse and fine nerve fibers and blood capillaries lie, apparently unaffected by the freezing (at -5° to 25°C.), surrounded by the network. The pink stained portion of the reticulum appears to be composed of the most delicate nerve fibers and condensations, as it were, of the tissue fluids; in fact, it may be regarded as being composed of all the acidophilic substances of the tissue. The blue stained portion, on the other hand, may be looked upon as composed of the basophilic sub-

stances of the tissue, especially the chromatin. The relation of the pink and blue portions to each other, and to the different conditions of the technique, will be considered more in detail under subsequent divisions.

In these stained preparations the various kinds of cells may be clearly distinguished. Red and white blood cells and glia cells appear, at temperatures above -25°C . quite unaffected by the freezing, excepting of course those that have been crushed or otherwise distorted in making the smear. Such crushed forms produce areas of coarser network. At lower temperatures (-25° to -40°C .) these cells, and the nerve cells as well, show a considerable variety of changes which will be discussed under the headings following.

c. Nerve cells of smears frozen at -5° to -10°C . The multipolar nerve cells of smears frozen at the temperature stated present appearances that vary somewhat depending, in addition to the reduction in temperature, on the thickness of the smear, the rapidity of the freezing, and no doubt also on the surface tension and other intrinsic forces of the different cellular elements. The size of the cell does not appear to play so great a part in these variations as one might expect. The change which is the most marked and characteristic of this temperature interval, and, in fact, the first change to become noticeable in the cells, is in the Nissl's bodies which become minutely reticular or vacuolated, thus forming many small blue-staining networks, each network corresponding to a single Nissl's body. These networks are loosely connected together by finer trabeculae of the same blue-staining substance (fig. 3). The trabeculae appear to be direct extensions of the Nissl's substance which has probably a lower freezing point. By virtue of this property these delicate processes have been squeezed out into the surrounding cytoplasm under the force of the displacement brought about by the enlarging and expanding ice masses. None of the networks extends beyond the walls of the cells. Aside from the Nissl's bodies, the cellular cytoplasm, the nucleolus, and the other nuclear structures show as yet no change, unless the nuclear chromatin, exclusive of the nucleolus, may be said to be beginning to suggest

reticular arrangement. Though this description applies particularly to smears frozen at -5° to $-10^{\circ}\text{C}.$, it also applies in part to smears frozen at temperatures as low as $-25^{\circ}\text{C}.$

d. Nerve cells of smears frozen at $-10^{\circ}\text{C}.$ and lower. The changes next in occurrence to those described under the heading just preceding are to be observed in cells of smears frozen at -10° to $-25^{\circ}\text{C}.$ In such preparations some of the cells show many of the Nissl's bodies still appearing as individual networks but with larger meshes and more closely interconnected, all however being still confined within the cell wall (fig. 4). In the cellular cytoplasm no changes are yet to be noticed. The nuclear chromatin (and also the achromatic substance of the nucleus which stains pink with erythrosin) has become distinctly reticular, apparently forming one continuous network limited to the area within the nuclear membrane; or, extending beyond the nuclear membrane and becoming continuous with the blue-staining network derived from the Nissl's bodies. The nucleolus may be unaffected, or may take part in the formation of the nuclear chromatin reticulum, forming as it were its starting point by sending out a varying number of processes. Some nerve cells show these changes much intensified with the cellular cytoplasm, exclusive of Nissl's bodies, also becoming reticular. This cytoplasmic reticulum, although continuous with the blue-staining network, is readily distinguished, when once fully formed, by the fact that it stains pink with the counterstain employed. In general, too, this pink-staining cytoplasmic reticulum has smaller meshes than the blue-staining network, and lies within the interstices of the latter. In order to avoid confusion, only the blue-staining portion of the reticulum is shown in the figures. Still other cells at this temperature ($-25^{\circ}\text{C}.$) and at lower temperatures (-25° to $-50^{\circ}\text{C}.$) show a reticulum with much larger meshes. The elements composing this reticulum are none other than those already mentioned, namely, the blue-staining network derived from the Nissl's bodies, nuclear chromatin, and nucleolus; and, the pink-staining network derived from the cellular cytoplasm (exclusive of Nissl's bodies) and the achromatic substance of the nucleus. The continuity of these two networks is one of

intimate blending and so a single double-staining reticulum results. This single reticulum extends throughout the entire cell and even beyond (fig. 5), and thus becomes directly continuous with the pink-staining extracellular network previously described under the observations on the stained smear as a whole. In these cells there is of course a complete obliteration of the Nissl's bodies as such. Thus, to repeat, it appears quite evident that the intracellular networks are directly continuous with each other, and again, are similarly continuous with the network formed in the cellular interstices. These last changes described may be called the final form the networks assume, for the appearances just described are quite constant and do not change to any noticeable extent in a temperature range of 20° to 30° (-40° to -60°C.). It cannot be said that any of the appearances described have an absolutely definite temperature at which they appear. For instance, the last form described may even be found to a certain extent in smears frozen at -15°C. , none of the earliest forms, however, occurs as a rule at temperatures below -25°C.

The significance of the networks described above and their relation to Möllgaard's reticulum or 'glia-network' will be discussed later.

ADDITIONAL OBSERVATIONS

The perivascular network described by Möllgaard may also be satisfactorily studied in the foregoing preparations. Its origin is plainly from the chromatin of the nuclei of the endothelial cells of the blood and lymph capillary and precapillary vessels (fig. 6). The variations and various relations of the network merit no description. The chromatin of the nuclei of the glia cells also form networks but their relation to both the networks of the nerve cells and the perivascular networks seems to be merely one of accident. The rather constant appearance of what Möllgaard believes to be a glia cell at one pole of the nerve cell nucleus the author did not observe and it is probably explained as being a modification of a chromatin nuclear cap.

All the observations on frozen neural tissue apply to smears frozen and fixed as soon as thirty-two seconds after decapitation

and at various intervals thereafter up to twenty-five hours post-mortem. After this time no further preparations were made, for it seemed quite evident that the networks were in some way related to the process of freezing rather than to the length of time after death. In this regard the author was entirely unable to confirm Möllgaard's statement that the earliest form of the reticulum (in nerve cells of tissue excised from the living animal) consists of a network of 3 to 4 meshes, and that the reticulum becomes finer and reaches its greatest development ten to twelve minutes post-mortem. The reticula of nerve cells in smears frozen and fixed thirty-two seconds after decapitation, and this time probably does not correspond very unfavorably with the time consumed by Möllgaard in excising and transferring the tissue from the living animal, were found as fine and as extensive as the reticula of cells in smears frozen and fixed at various later intervals up to twenty-five hours after death. It seems apparent, therefore, that very little, if any, connection exists between the fineness and extensiveness of the reticulum on the one hand and the time after death, up to at least twenty to twenty-four hours, on the other.

Neither was the author able to note any disintegration of the reticulum in nerve cells of frozen smears left in 96 per cent alcohol for a time varying from half-an-hour to three days. Even when Möllgaard's modification of his technique, as previously explained under 'Material and technique,' was carefully followed out, as nearly as the technique and conditions of the present investigation would permit, no such changes as noted by Möllgaard were observed in the nerve cells of the smear preparation.

Alterations in the staining properties of the protoplasm, as observed by Möllgaard in frozen preparations made at various intervals after death, were given but little attention because smear preparations do not lend themselves readily to a reliable study of such details. Furthermore, very much depends on the thickness of the smear and its degree of differentiation after staining. It may be noted however that in smears frozen twenty-four to twenty-five hours after death there is a more diffuse staining with the basic stain in consequence of which the blue and

pink staining networks become less distinct and in places ill-formed.

That post-mortem changes have a marked influence on the cytological appearance of the tissue there can be no question. But it is questionable if the post-mortem changes produce alterations sufficiently great to be detected by our present technique until a considerable time after death, at least half-an-hour. The relationship between the acid reaction of the neural tissue and the post-mortem changes produced are not so strikingly noticeable as Möllgaard's emphasis on this point would lead one to believe. Möllgaard states that the cerebrum of dog shows a marked acid reaction to moist neutral litmus paper ten minutes after death. It was however fully twenty minutes before the author could be sure of such acid reaction. Nevertheless, it is quite possible that with a more delicate indicator an acid reaction might be observed somewhat earlier.

To determine whether the network could be produced in cells that had first been fixed, some smears were made approximately seven minutes after death, fixed in 96 per cent alcohol at room temperature for one hour, and then subjected to a temperature of -15°C . At this temperature (which was possibly not low enough) no networks could be observed in the cells. The Nissl's bodies had remained unchanged. This question of the possibility of structural change through freezing after fixation, however, was insufficiently investigated to warrant any definite conclusions; yet, it is a matter of common knowledge that in the use of various neurological methods where the tissue is frozen after fixation no *marked* changes at least have been reported.

Smears of fresh hepatic and pancreatic tissues, prepared after the manner of the neural tissue preparations, show networks which are markedly similar to those of the neural tissue, the chromatin of the nuclei giving rise to a blue-staining network while the non-chromatic elements give a pink-staining network. Thus it may be said that Möllgaard's blue-staining reticulum is not a characteristic of neural tissue alone but may be formed from the chromatin of the nuclei of hepatic and pancreatic cells as well.

The networks in the liver and pancreatic tissues, as well as those of frozen smears of egg-albumen, may also be stained with silver nitrate.

In corroboration of the foregoing observations on animal tissues it may be mentioned that Molisch has observed very similar appearances in the freezing of amoebae, fungi, yeasts, and various algae. Molisch's observations however apply principally to cytoplasmic changes. Equally instructive and showing chiefly the nuclear changes due to freezing are the numerous figures of Matruchot and Molliard ('02) in their work on the influence of freezing on plant cells.

The observations on the neurofibrillae, like those on the Nissl's bodies, may be considered under two headings; namely, their presence in the unfrozen and in the frozen cells. The neurofibrillae are unquestionably present in the unfrozen cells of smears fixed twenty-five seconds after decapitation and thereafter until lost in the total disintegration of the tissue. Although the perinuclear network and other finer details of the endocellular neurofibrillar structure of the nerve cell (such as may be seen in thin sections) are not visible as such in smear preparations, the neurofibrillae are distinctly seen at the origin of the cell processes and an endocellular neurofibrillar network of varying richness is more or less discernible in the cell-body.

In regard to neurofibrillae in frozen preparations it may be noted that smears frozen at -5° to -20°C . fixed in 96 per cent alcohol, and impregnated with 1.5 per cent silver nitrate for from nine to twenty-one days, show networks corresponding more or less closely to the networks stained with toluidin-blue and erythrosin. Large numbers of darkly stained nerve fibers render the appearance somewhat difficult of recognition at first sight, especially where the smears are thick. In the larger meshes of these networks lie the nerve cells whose individual reticular structure may or may not be continuous with the walls of the meshes about them. In these preparations, when not too darkly stained, the neurofibrillae are unmistakably present in the nerve cells and their processes as well as in the intercellular nerve fibers. They are well stained and quite distinct. The neuro-

fibrillae however are best observed in preparations frozen quite rapidly and at moderately low temperatures (-5° to $-15^{\circ}\text{C}.$), for when thus treated the intracellular neurofibrillae do not seem to take part in the formation of the cellular reticulum corresponding to that stained with toluidin-blue and erythrosin. At temperatures below the foregoing, especially if frozen slowly, the intracellular neurofibrillae cannot be made out with any degree of certainty though they are still quite distinct in many of the cell processes and in the fine nerve fibers of the intercellular spaces. The above observations were made on smears frozen and fixed forty-two seconds after decapitation and at different times afterwards up to twenty-five hours after death. These observations on the neurofibrillae in frozen tissue are quite in agreement with Liesegang's ('11) conception; but, on the other hand, quite opposed to that of Auerbach ('11).

In the above study of neurofibrillae both in the unfrozen and the frozen preparations many variations were met with. In this regard, as Legendre ('06), Marinesco ('09), and many others have noted, it may be mentioned that the methods for the demonstration of neurofibrillae are not sufficiently adequate or reliable to permit of constant results. Only by extensive observation and many controls can definite conclusions be reached. So it may be questioned whether the above observations on the neurofibrillae in frozen nerve cells, in this one investigation, are sufficient to determine the point at issue; yet, since this point is merely the presence of the neurofibrillae in such frozen cells, without reference to the details of their distribution, and so forth, the author believes the results may be regarded as quite reliable.

It may be of interest to note that both the Nissl's bodies and the neurofibrillae were unmistakably present in nerve cells from the cervical portion of the spinal cord of horse (cf. 'Material and technique'), fixed three minutes after the instant of the shooting of the animal.

In imbedded tissue, that is, tissue fixed, dehydrated, imbedded in paraffin (or celloidin), cut, stained, and mounted; both the Nissl's bodies and the neurofibrillae were clearly and distinctly

present in cells fixed *twenty-two* seconds after decapitation. Small pieces of the cervical portion of the spinal cord of dog were quickly dropped in the fixative at the time stated. Then in the cutting, staining, and mounting of the sections care was taken to use only those sections cut from the most superficial parts of the tissue block. Thus one may be reasonably sure that the fixation of the superficial cells was practically simultaneous with the committal of the tissue to the fixative.

No observations were made on imbedded specimens of frozen tissue.

DISCUSSION

It was one of Möllgaard's primary objects to produce a method which would be simpler than our present neurocytological methods. His procedure however is not so strikingly simple as he would have us believe. In the first place, the production of a temperature as low as $-40^{\circ}\text{C}.$ and the necessity for maintaining it for a definite length of time requires a painstaking and rather cumbersome technique. Sectioning the tissue at a temperature of -20° to $-15^{\circ}\text{C}.$ at best, and that with a specially constructed microtome, is not easy. Even in the fixation, if the author has correctly interpreted Möllgaard's following statement, complicating factors enter: "Die Flüssigkeit, in der man zu schneiden wünscht, wird in den innersten Kasten des Kalorimeters gegossen und durch Zusatz von fester CO_2 auf eine Temperatur von -20° bis -25° heruntergebracht." Judging from the description of his apparatus this seems an unnecessary step, yet he so directs us. Bohr (Annalen der Physik, IV F, Bd 1, S. 244, 1900) states that at -20° and $-40^{\circ}\text{C}.$ (760 mm.?) 98.7 per cent (by weight, 15° , 760 mm.) ethyl alcohol will absorb 7.16 cc. and 13.89 cc., respectively, of CO_2 (0° , 760 mm.). In 96 per cent (the percentage used by Möllgaard) alcohol the absorption is of course less. However, Möllgaard thus leaves without control the possible changes the tissue may suffer from the effects of the CO_2 . At the temperature stated it may be questioned if there is any effect on the tissue, still it cannot be nil. The presence, and its liberation as the temperature rises, of the CO_2 in the fixative does not simplify the technique to say the least.

Finally, his method requires the preparation of the animal, its anesthetization, the performance of a craniotomy or a laminectomy, and the waiting for the effects of the anesthetic and the shock of the operation to disappear. In connection with the latter features, it may be well to mention that Dolley ('09 and '10) has conclusively shown that the effects of the anesthetic and the shock of the operation do not disappear in so short a time (seven to eight hours) as Möllgaard assumes. So that for various reasons, in addition to that urged by Retzius, Möllgaard's technique is by no means ideal.

It was in order to avoid the foregoing objections that the technique previously described was decided upon for the present investigation. The decapitation avoids all preliminary preparation of the animal, and likewise excludes the effects of an anesthetic and the prolonged shock of the operation. The apparatus for the decapitation permits one to obtain practically instantaneously a segment of the animal's neck, after which the isolation of the cord, with a little experience, requires but a few seconds. By resorting to smear preparations, the freezing process, which the sectioning of an unimbedded tissue demands, is avoided. The smear preparations have the further advantage of being fixed the very instant they are consigned to the fixative. Thus it is evident that the entire procedure, namely, the taking of the tissue, putting the tissue in a form suitable for study, and the fixation, all occur in less than a half a minute of time, or at most a minute. It should be noted that the cumbersome freezing method of Möllgaard is designed merely to preserve the tissue structure in the 'vital condition' until thin sections can be made upon which the fixative can act. The smear method accomplishes this far more easily and quickly, and eliminates the production of artefacts by freezing. It is of course freely granted that the smear preparations do not show the minute details of structure as readily as do thin sections. Nevertheless, for the points in question the smear preparations are amply sufficient.

In smear preparations not subjected to the freezing process the Nissl's bodies and the neurofibrillae are unquestionably pres-

ent twenty-five seconds after decapitation. It has been previously mentioned that in nerve cells of imbedded tissue the Nissl's bodies and neurofibrillae were found in tissue fixed twenty-two seconds after death. It has been stated too that the smear preparations do not permit of the detailed study that sections do. It is therefore unnecessary to discuss the various details of observation noted under this heading. It is particularly the time element with which we are here concerned. Möllgaard found no Nissl's bodies or neurofibrillae in freshly-frozen and fixed nerve cells. He considers at least seven minutes post-mortem change, followed by several hours of slow alcohol fixation, necessary to produce the Nissl's bodies even imperfectly. But the results submitted in the present paper prove that in unfrozen smear preparations (which have the advantage of excluding the production of artefacts by freezing and allow immediate fixation) both the Nissl's bodies and the neurofibrillae are found in tissue fixed less than half a minute after decapitation, while the cells are still practically in a living condition. Hence Möllgaard's contention that Nissl's bodies are produced by post-mortem change and slow alcohol fixation is totally wrong. The freezing is responsible for his misleading results.

Whether the sojourn in the fixative be comparatively long or short seems to have no noticeable influence on the Nissl's bodies. In smears taken at various intervals after death the Nissl's bodies show no appreciable change till about twelve to eighteen hours post-mortem, when they gradually begin to disintegrate. To determine whether the alcohol fixation could in any way be responsible for the presence of the Nissl's bodies in the freshly-fixed cell, some smears, instead of being dropped into the fixative, were immediately consigned to the toluidin-blue stain, after which they were carefully washed, mounted in water, and studied. Such smears show the Nissl's bodies to be undeniably present. They show distinctly, but it seems that they are somewhat more granular and possibly a little more diffuse than those of cells fixed in alcohol. In this connection it may be mentioned that Dogiel ('96) stained the Nissl's bodies in unfixed nerve cells, with dilute solution of methylene-blue in warm physio-

logical salt solution five to ten minutes after death. It may be possible however, as Held ('95) has pointed out, that the toluidin-blue or the methylene-blue (Dogiel) may fix, or partially fix, the tissue put into it.

Regarding the alterations which freezing produces in the smear preparations, very much depends upon the conditions under which the freezing occurs. The factors previously referred to may be repeated, namely, the composition of the substance or tissue in question, the thickness of the smear, the degree of temperature reduction, the rapidity of the freezing, the surface tension and other intrinsic forces of the individual elements of the substance or tissue investigated (e.g., the forces of molecular attraction, imbibition, osmosis, etc.), to say nothing of thawing and fixation. These factors have been wholly or partially recognized by all investigators of the phenomenon of freezing.

The freezing of water has been sufficiently discussed. The physico-chemical explanation of ice-formation, though of fundamental importance in the following considerations, are given in detail by Quincke and so will be referred to here only as occasion demands.

The appearances observed in the freezing of egg-albumen are concisely expressed in Molisch's following statement in which he describes his microscopical findings for the freezing of a 2 per cent aqueous solution of gelatin:

An zahlreichen Punkten tauchen unter Abscheidung von Luftblasen rundliche Eismassen auf, die, der benachbarten Gelatinegallerte das Wasser entziehend, sich rasch vergrössern und dabei die immer wasserärmer werdende Gelatine ringsum zur Seite schieben, so das diese, wenn die Eisbildung ihr Ende erreicht hat, als ein höchst complicirtes Maschenwerk zwischen den Eisklumpchen ausgespannt erscheint. Die ursprünglich homogene Gelatine ist nun in eine Art Schwamm umgewandelt, in welchem das höchst complicirte Gerüstwerke aus Gelatine, die Hohlräume aber aus Eis bestehen.

Molisch's observations on frozen egg-white are similar to his above observations on gelatin, excepting that in the case of the egg-albumen the network disappears on thawing, while that produced in gelatin is quite permanent for some time. It is of interest here to note that Ambronn ('91) in his study of frozen gelatin and agar-agar states that the appearance of the fine network

due to the freezing of dilute solutions of these substances is exactly similar to the appearance of a section through a parenchymatous plant tissue. Molisch casually remarks that his studies confirm this statement. Furthermore, Ambrohn states that in an optical respect the walls of the meshwork in the frozen colloids named are entirely similar to the walls of plant cells, showing a strong double refraction and the same orientation of the optical elasticity ellipsoid as do the cell-walls.

From the observations and references presented, it must be clear then that on freezing there is a separation of water from the substance in question. This fact has long been known to many observers (Müller-Thurgau, Molisch, Fischer, Wiegand, and others). The water separating out under the reduced temperature forms ice, which, omitting for the present the details, by displacement of the substance produces the network or sponge-like structure described. The great part played by this water separating out during the freezing of the substances and tissues under consideration, may be further realized by a study of smears of egg-albumen, fresh neural, liver, and pancreatic tissues thoroughly evaporated or desiccated in an oven. Such preparations show networks somewhat suggestive of those produced by freezing. So similar are the processes of freezing and desiccation, in producing a loss of water, says Fischer, that the curve for the loss of water by freezing may be calculated, for some colloids at least, from the curve for the loss of water by desiccation. Matruchot and Molliard have compared the separation of water during freezing to plasmolysis; and, wilting, or slow and rapid desiccation, as well.

Noting the comparative uniformity of the results arrived at by many observers regarding the fundamental principles underlying the freezing of numerous simple substances, colloids, and plant and animal tissues we may consider, as does Molisch, and with considerable support, that the cells of a plant or animal tissue may be regarded, as far as their behavior in freezing is concerned, as aggregate masses of solutions, emulsions, and colloids. Thus, for explanatory purposes, we may consider that neural tissue with all its various elements consists of a complex aggregate of solutions, colloids, and emulsions, one within the

other, most delicately interrelated and adjusted, specialized and differentiated, if such terms are permissible in this connection, to a high degree. There is however the one common element, water, whose proportional presence depends on the various conditions to which the tissue may be heir. If now this complex aggregate be subjected to a freezing temperature, there is, as is already evident from previous statements, a separation of water. This separation of water is strikingly dependent on the factors before noted. If the freezing is slow the water as a rule collects in the interstices of the tissue and thus in the subsequent ice formation there are comparatively few centers of crystallization. If however, the freezing is rapid the rapidity of the process does not permit the water to collect in a few, apparently the least resistant, interstices but forces it to crystallize in numerous places. It would seem that in the employment of low temperatures, the more rapid the reduction the more numerous the centers of crystallization. Liesegang states that if the reduction in temperature be great enough the centers of crystallization become so numerous as to warrant the designation colloidal ice, the existence of which has been proven by Ostwald and Weimarn (cited by Liesegang).

Since it is in the more slowly frozen tissue that the more typical reticular structure occurs, we must return to that condition. Just how and why the water of the various tissue elements collects in the interstices of the tissue during the process of freezing is explained in considerable detail, in the case of plant tissue, by Wiegand ('06 b). Just what, at a freezing temperature, determines the formation and location of a center of ice crystallization Wiegand does not state. Many factors no doubt are involved, the more important ones however are probably the following: the minimal amount of solute present in the water, and the relative molecular capillarity with which this water is held, together with the molecular distribution and arrangement, at that particular instant and position. With the formation of the ice crystals of course comes the molecular force of crystallization which continues to abstract water from the particular tissue element in question with a simultaneous increase

in the size of the ice crystal, or crystals. This process of abstraction of water and enlargement of ice crystals continues until the force of crystallization is equal to the force of imbibition, or molecular capillarity, of the tissue element or cell, that is, until an equilibrium is reached. With a new reduction in temperature the process is again set up until the equilibrium is once more restored.

Whatever the exact details of the process may be, it is evident that with the first formation of the center of ice crystallization the tissue elements, and cellular elements especially, are subjected to a displacement which is increased in extent both by the expansive force of the water changing to ice and the actual increase in size of the ice crystals under the force of crystallization. With a moderate rate of freezing (such as making smears on slides cooled to -20° to $-40^{\circ}\text{C}.$, with fixation at the same temperature) and a like temperature reduction this displacement gives rise to the various networks or sponge-like reticula described for the egg-albumen, nerve cell, and so forth. These reticula are therefore the resultant of aqueous abstraction, and displacement of the subsequent tissue or cellular residue, as it were, by the formation and growth of ice crystals or ice masses within the tissue or cell. The expansion and contraction of the ice, as the maximum and minimum temperatures for these phenomena are reached and surpassed, augment the displacement, while the force of imbibition is probably the main retarding force. With very rapid freezing at very low temperatures ($-50^{\circ}\text{C}.$ and below) the formation of multiple centers of crystallization subjects the individual cellular elements to the contraction of the frozen mass of course much more than at higher temperatures. That the networks discussed arise in consequence of the displacement by the enlarging ice masses is particularly emphasized by Müller-Thurgau, Molisch and Wiegand, as before stated.

The blue and pink staining properties of the reticulum are explained as being due to the fact that some of the substances composing it are basophilic while others are acidophilic, that is, some of the composing substance stains with toluidin-blue while other portions of it stain with erythrosin. If now, we regard

the Nissl's bodies as composed of substances of the nature of chromatin, as is generally done, then we may say that the blue-staining (with toluidin-blue) networks or reticula are derived from the Nissl's bodies and nuclear chromatin of the nerve cells as well as the nuclear chromatin of all other cells present in the preparation. The pink-staining (with erythrosin) networks, on the hand, may be regarded as arising from all the achromatic substances of cellular cytoplasm and tissue (blood, lymph, etc.) alike. That such is indeed the origin of the networks or reticula is confirmed by this investigation. Möllgaard's reticulum therefore arises from the Nissl's bodies and nuclear chromatin. The reticulum is a product of the Nissl's bodies rather than the converse as believed by Möllgaard. The close relationship between the reticulum and the Nissl's bodies is further supported by the many transition forms that may be noted in nerve cells of smears frozen at moderately low temperatures (-5° to $-25^{\circ}\text{C}.$) (figs. 3 and 4).

Retzius in his study of the freezing of various tissues and fluid masses lays much stress on the formation of a system of clefts and lacunae due he says to the collection of the water at the moment of freezing at the points of lowest resistance. The formation of ice in this system produces distortion and laceration. With fixation the entire picture is preserved and is known to us as the artefacts due to freezing. Excepting for the undue stress laid on the formation of a system of clefts and lacunae and the production of real laceration, it is evident that Retzius' results are in the main quite similar to those arrived at in this investigation. The cleft and lacunar system of Retzius, however, must not be confused with the clefts spoken of here as due to the contraction of the ice present.

CONCLUSIONS

From the observations presented and the discussion made the following conclusions may be summarized:

1. With a simplified smear method, both the Nissl's bodies and the neurofibrillae are found present in the spinal nerve cells of the dog, fixed *twenty-five* seconds after decapitation.

There is no evidence that they are artefacts due to post-mortem changes as described by Möllgaard.

2. Nissl's bodies and neurofibrillae may also be demonstrated, in a more or less modified condition, in frozen neural tissue. The freezing causes the Nissl's bodies and nuclear chromatin to assume the form of a reticulum. This reticulum is identical with Möllgaard's reticulum, or 'glia-network.'

3. Möllgaard's reticulum is produced during the process of freezing, and is due to the displacement incurred by the enlarging and expanding ice-masses which form in the cell or tissue at the reduced temperature.

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PLATE 1

EXPLANATION OF FIGURES

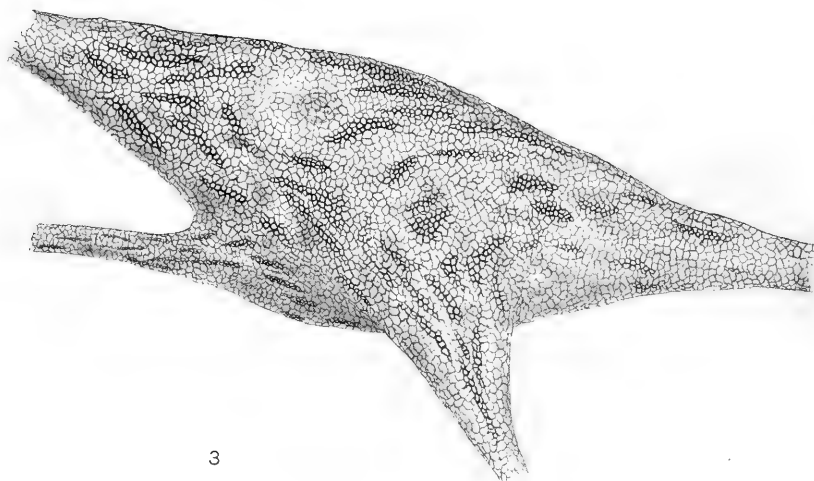
Figures 2 to 6 were all drawn with the aid of a camera lucida. Figures 2 to 5 are anterior horn cells of spinal cord of the dog, from smears fixed in 96 per cent alcohol and stained with erythrosin and toluidin-blue. In the case of the frozen preparations only the blue-staining reticulum is represented, with this exception, that in figure 5 a small portion of the pink-staining extracellular reticulum is represented to show its continuity with the blue network. The individual differences will be noted in connection with the separate legend for each figure.

2 Anterior horn cell of an unfrozen smear, fixed twenty-five seconds after decapitation. The Nissl's bodies are distinctly present. Magnification 750 diameters.

3 Anterior horn cell, frozen and fixed at about -20°C ., seventy-four seconds after decapitation. The figure represents one of the early transition forms, the Nissl's bodies appearing as minute networks loosely joined together. Magnification 750 diameters.



2



3

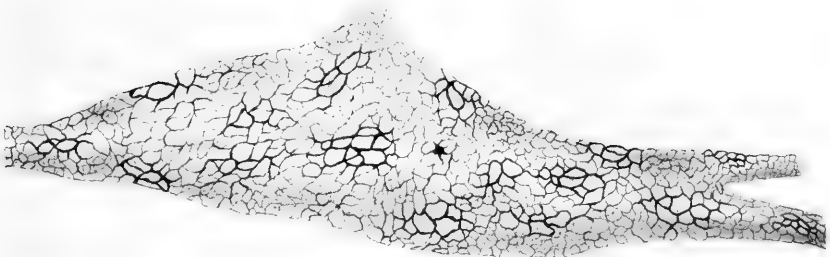
PLATE 2

EXPLANATION OF FIGURES

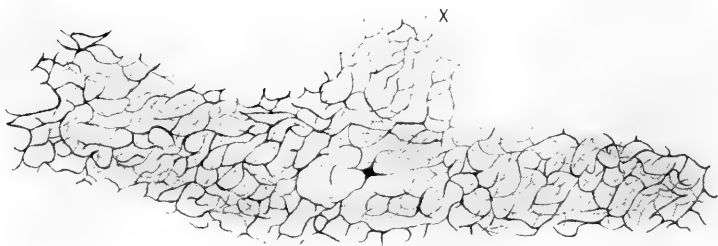
4 Anterior cell, frozen and fixed at about $-25^{\circ}\text{C}.$, one minute and forty-three seconds after decapitation. This is a more advanced stage than figure 3, but there are still evident traces of the Nissl's bodies from which the network was derived. The nucleus is faintly discernible. Magnification 400 diameters.

5 Anterior horn cell, frozen and fixed at about $-25^{\circ}\text{C}.$, one minute and forty-three seconds after decapitation. From the same smear as figure 4. This represents one of the extreme forms in which practically all evidence of the former Nissl's bodies is lost. In the area marked X there is observed a small portion of the extracellular pink-staining network which is continuous with the intracellular blue-staining reticulum. This area in this figure is the only place where any of the pink-staining network is shown. The nucleus is represented by the area having but few meshes. The nucleolus is much distorted. Magnification 600 diameters.

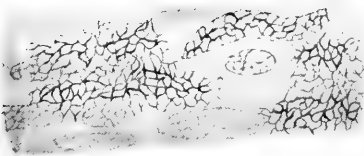
6 A somewhat diagrammatic representation of Möllgaard's 'perivascular network' in the walls of a precapillary vessel. Frozen and fixed at about $-40^{\circ}\text{C}.$, forty-two seconds after decapitation. From a smear of neural tissue. Note that some of the networks extend beyond the nuclear walls while others are still confined within them. In those extending beyond the limits of the nucleus the nuclear wall is apparently lost in the network. Magnification 1000 diameters



4



5



6

THE STUDY OF AN ATYPICAL CEREBRAL CORTEX

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NINE FIGURES

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INTRODUCTION

In a previous paper (2) I have described briefly some of the histological findings in a quite atypical cerebral cortex from a cyclopiian term foetus. In the present communication it is my intention to deal with the structure of various areas in the same cortex somewhat more fully, for it seems to me that these details are warranted in view of the manner in which the cortical development in this case illustrates several rather fundamental theories concerning the growth of nervous tissue.

The cerebrum in this case was represented by a single unpaired vesicle, having an extensive expanded ependymal roof which was attached to the recurved margin of a cup-shaped thickened base representing the cortex proper. The attachment of the cerebral vesicle to the thalamus was very slight and wholly basal. On

this account projection fibers of thalamic origin encountered considerable mechanical difficulty in gaining the cortex. Many of these fibers failed to reach the cortex and of those that did, the majority come to an end within a short distance of their entrance. These somewhat complicated relations are fully described in the paper cited above.

The thickened basal portion of the cerebrum representing the cortical area was divided by a Y-shaped median furrow into a median anterior and two lateral posterior regions or lobes. This furrow is not of morphological significance and represents only a mutual form adaptation between the cerebrum and skull base. However, for purposes of description I shall refer to these regions outlined by this furrow as lobes. The relations obtaining in this brain may be seen on reference to figures 1, 2 and 3. In these figures are indicated the approximate areas from which the sections here illustrated were taken.

The drawings of these sections were all made with the aid of a Leitz projectoscope at a magnification of 130 and are reduced to $\times 65$ in reproduction. This magnification was selected as being the lowest at which even the approximate shape of the smaller cells could be shown. The whole thickness of the inner cortical stratum is not indicated in any of these drawings.

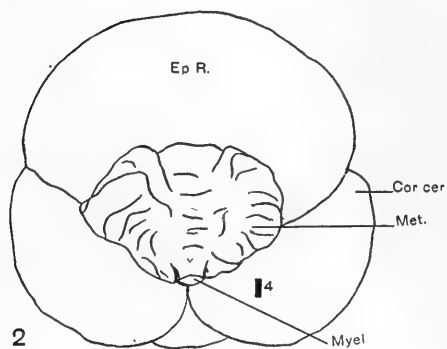
Technique

Modified Weigert, modified Nissl and simple hematoxylin and eosin stains were used. The tissue was not in a favorable condition to react to metallic impregnations and although numerous attempts were made, no successful preparations were obtained.

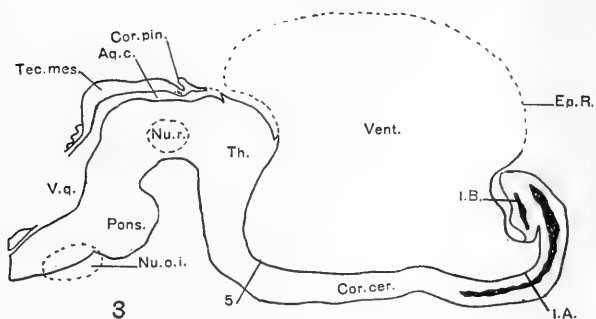
The approximate distribution of the thalamic fibers, which were the only medulated fibers present in the cortex, was determined by a modified Weigert method. It was found that these fibers when present coursed in general, parallel to the surface of the cortex. No typically radial fibers were found. The fibers were poorly medullated and gave to the tissue a peculiar, coarse, reticulated character quite different from the almost homogeneous appearance of the fiber laminae of the cortex proper.



1



2



3

Fig. 1 Outline drawing of ventral view of whole brain. *Cor. cer.*, cortex cerebri; *Ep.R.*, ependymal roof; *Met.*, cerebellum; *M.F.*, Y-shaped median furrow; *Myel.*, medulla; *P*, pons. The approximate areas from which the sections drawn were taken are indicated by their respective numbers.

Fig. 2 Outline drawing of entire brain—posterior view. Lettering as in figure 1. The region on the posterior pole of the right posterior lobe from which the drawing for Area 4 was taken is indicated by the numeral.

Fig. 3 Diagram of a median sagittal section of the entire brain. *Aq.c.*, iter; *Cor. pin.*, pineal body; *Nu.o.i.*, inferior olive and *Nu. r.*, red nucleus; the position of these nuclei are indicated by dotted lines; *Tec. mes.*, midbrain roof; *Th.*, thalamic mass; *Vent.*, cavity of forebrain vesicle; *V.q.*, fourth ventricle; other lettering as in figure 1. The approximate areas from which the sections drawn were taken are indicated by their respective numbers.

DESCRIPTION OF CORTICAL AREAS

Over the major portion of the cortex the cellular elements are arranged in such a way as to form five quite definite strata. These are: I, an outer or plexiform layer well marked over all areas; II, an irregularly arranged cell layer; III, a layer sparsely supplied with cells and which I have compared elsewhere with Bolton and Moyes' (3) inner fiber lamina; IV, a layer of closely packed cells; and V, a very thick inner or polymorphic cell layer. In many regions this last layer tends to be subdivided into two strata described when present as layers V and VI.

After this general statement as to cell lamination, a more detailed description of certain definite areas will be given. The regions selected are as follows: (1) two different areas from the anterior median lobe; (2) an area bordering on the right anterior limb of the Y-shaped median furrow; (3) from the central portion of the base of the left posterior lobe; (4) from the posterior pole of the right posterior lobe; and (5) from the region of junction of the thalamus and cerebral vesicle.

Area 1 A. Cortex over the anterior basal portion of the anterior lobe. Average thickness 6.7 or 6.8 mm. (fig. 4).

Layer I. The zonal layer is thick, prominent and sharply marked off from the subjacent stratum. Occasionally there are found in the deeper parts of this layer irregular medium sized multipolar cells, but most of the cells found here are of embryonic character.

Layer II. A very irregularly arranged layer of cells showing but little differentiation. The elements tend to be arranged in compact more or less distinct groups. Not quite so thick as Layer I.

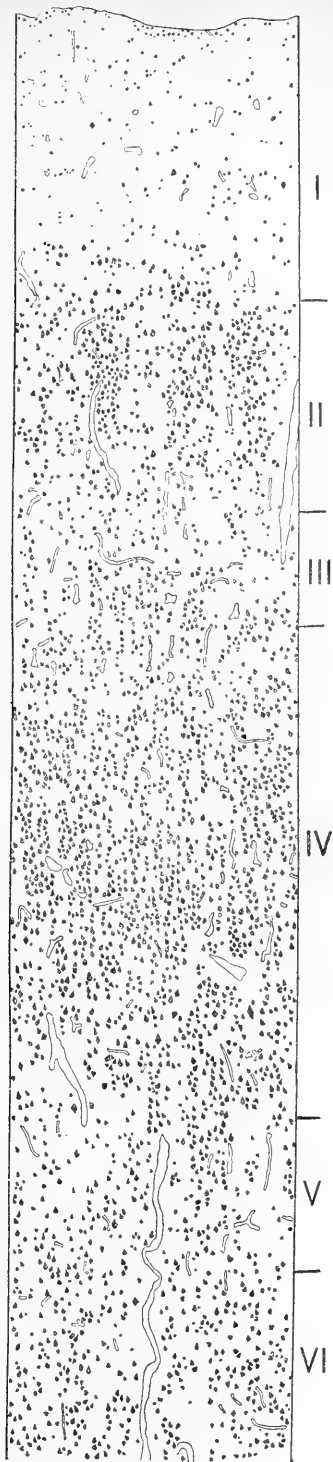
Layer III. A layer poor in cellular elements and of inconstant thickness owing to the varying depth of the superadjacent lamina. The line of demarcation between this layer and the subjacent stratum is fairly sharp.

Layer IV. A very thick and well marked stratum of closely packed cells. In the deeper portions of this layer there is some evidence of slight cellular differentiation. The majority of the small cells show a tendency

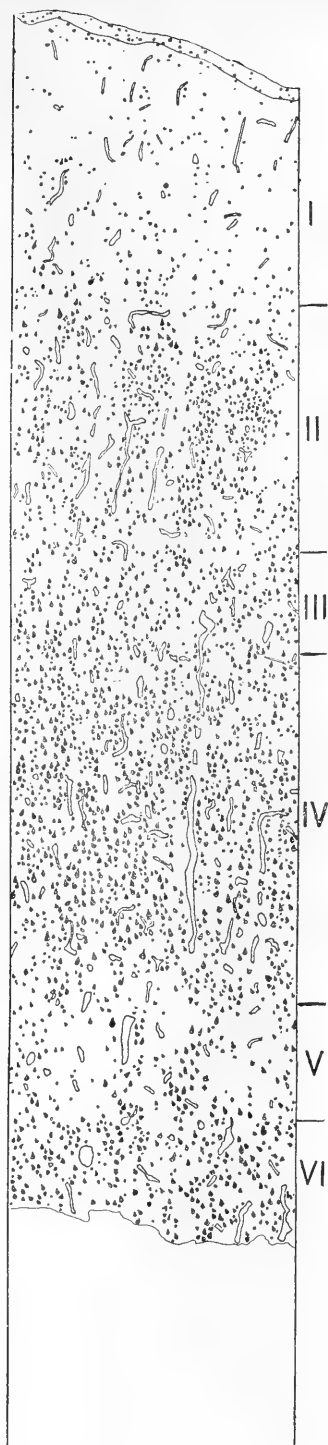
Fig. 4 Section through the cortex of the anterior basal portion of the anterior lobe. Area 1 A; this region is indicated in figures 1 and 3. $\times 65$.

Fig. 5 Section through the cortex over the inner pillar of the anterior recurved margin of the anterior lobe. Area 1 B, region indicated in figure 3. $\times 65$.

4



5



toward typical embryonic arrangement into rows at right angles to the surface of the cortex.

Layer V. A stratum of less densely packed cells which varies in thickness at the expense of the subjacent lamina.

Layer VI. An irregular stratum of varying thickness made up of polymorphic and embryonic elements arranged in groups of various sizes and on the whole showing a denser arrangement of cells than Layer V.

Area 1 B. Cortex over the inner pillar of the anterior recurved margin of the cerebrum. Average thickness 2.5 or 3 mm. (fig. 5).

The lamination occurring over this area is essentially similar in its arrangement to that already described for Area 1 A. The cortex is here however markedly thinner and cell differentiation is even less evident. In the zonal layer there are no medium sized multipolar elements to be found. At the cortical limbus all cell laminae blend as described in a previous paper (2). In the figure the inner or polymorphic layer is interrupted by a shrinkage cleft.

In both these areas described Layers V and VI are easily to be distinguished as separate strata. Below, Layer VI gradually merges with the medullary center.

Area 2. Cortex from the anterior part of the right posterior lobe. Average thickness 5.2 mm. (fig. 6).

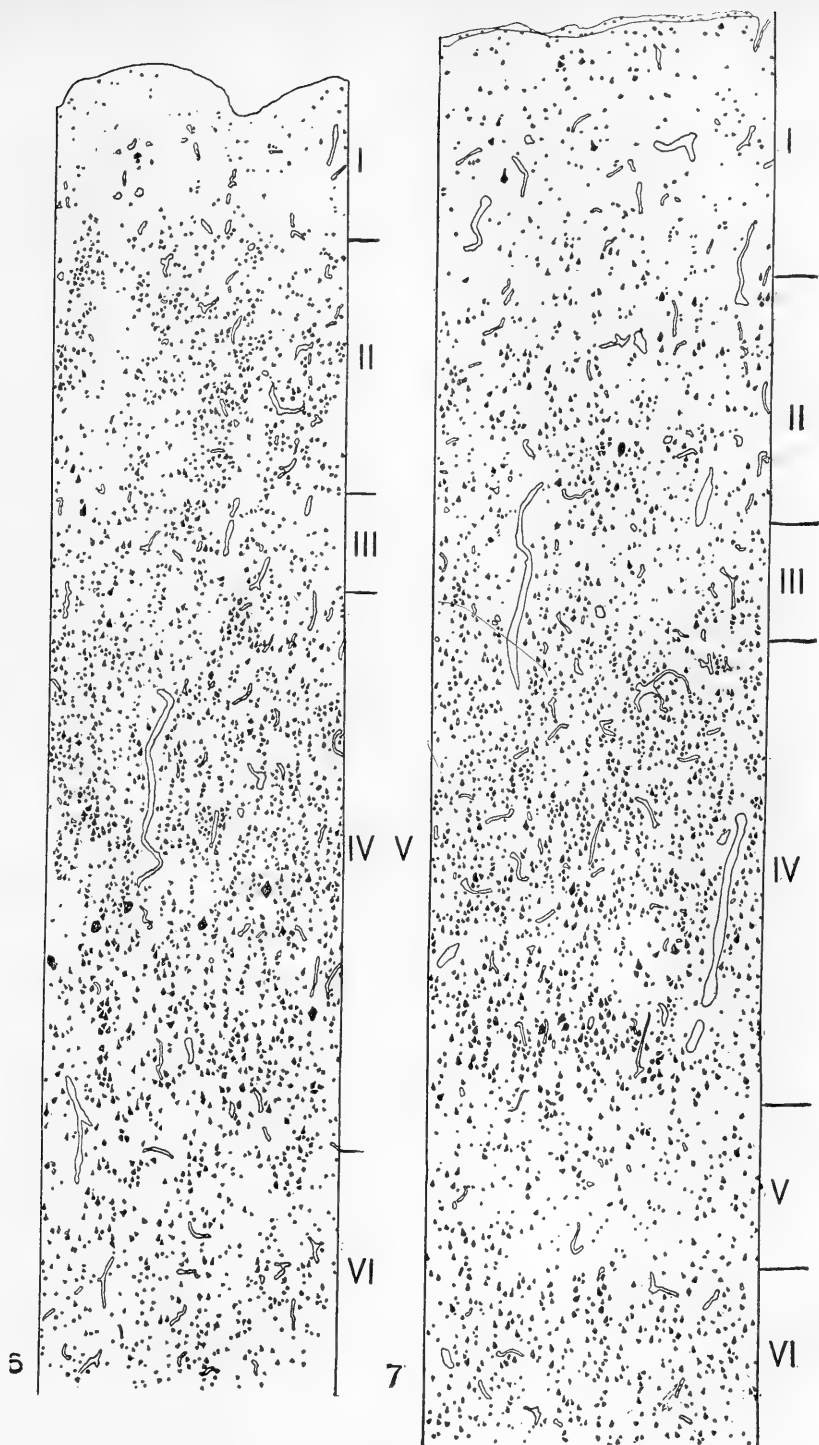
Layer I. Well developed but, as may be seen from the figures, it is much thinner than the corresponding layer in the sections described above. In the deeper portion of this stratum or in the upper parts of the subjacent layer there are found not infrequently quite large irregular multipolar elements. When seen they form a marked contrast to the small undifferentiated cells predominating at this level.

Layer II. This layer shows no essential difference from Layer II as described in Area 1.

Layer III. In some places in this region this layer is to be distinguished only with difficulty. Its presence depends upon the size of the irregular cell groups of the superadjacent layer, which at times almost blends with Layer IV. The cells are small and are mostly of embryonic nature. Occasionally at the junction of Layers II and III there are found medium sized pyramids fairly well differentiated.

Fig. 6 Section through the cortex bordering the right anterior limb of the Y-shaped median furrow. Area 2, region indicated in figure 3. $\times 65$.

Fig. 7 Section through the cortex from the central portion of the base of the left posterior lobe. Area 3, region indicated in figure 3. $\times 65$.



Layers IV and V. These two layers are so blended as to be indistinguishable in this region. Together they make up a well marked and densely packed cell stratum which varies in thickness considerably at different points. In its deeper portion there occur either singly or in groups, certain giant polymorphic cells averaging 36 by 43 microns in size.

Layer VI. The polymorphic layer is not sharply marked off from the layer above and the line of demarcation is in places very indistinct. This layer and the superadjacent stratum make up a good two-thirds of the total cortical thickness in this area.

Area 3. Cortex from the central portion of the base of the left posterior lobe. Average thickness 5 mm. (fig. 7).

Layer I. The zonal layer is well developed but not very sharply marked off from Layer II. Irregular quite large multipolar elements are of frequent occurrence in this stratum, which is otherwise similar to the corresponding lamina in the areas described.

Layer II. This layer is not so well developed as is the case in the more anterior portions of the same lobe. The crowding of the cells into irregular islands is not so marked. The most characteristic feature of this layer is the occurrence here and there of giant polymorphic cells averaging 33 by 40 microns in size.

Layer III. This layer is readily distinguished by the sparseness of its cell content but its boundaries are by no means definitely marked.

Layer IV. This layer is thick and well marked. The majority of the elements in the more superficial portions of the stratum are small and poorly differentiated. The characteristic arrangement into rows or columns is well shown. In the deeper parts of the lamina, numerous well developed medium and large pyramidal elements occur. This part of the stratum is quite evidently in a more advanced state of differentiation.

Layer V. Though seen in some places, as for instance in the area drawn in figure 7, this layer is to be made out only with difficulty in this region.

Layer VI. There is a tendency toward grouping of the elements of this layer into irregular islands as was the case in this stratum over the anterior lobe. Otherwise this layer does not differ essentially from the polymorphic stratum of other areas.

Area 4. Cortex from the posterior pole of the right posterior lobe. Average thickness 5.2 to 6 mm. (fig. 8).

Layer I. The zonal layer is very thin, being little more than one-third as thick as over other areas of the cortex.

Layer II. This layer is fairly well marked and is made up of medium and small sized polymorphic and pyramidal elements somewhat loosely arranged. In the outer portion of this stratum large polymorphic cells

are of frequent occurrence. The tendency toward arrangement of elements into island-like groups is not so marked as is the case in this stratum over other areas.

Layer III. Though not well developed, this layer can for the most part be distinctly made out and presents no special peculiarities.

Layer IV. This lamina is markedly developed and is somewhat thicker than all the strata superficial to it combined. It is made up of a dense collection of cells of embryonic, pyramidal and polymorphic types. The crowding of the elements here is not so great, however, as is the case in this stratum over the anterior lobe and there is a relative predominance of quite well differentiated cells.

No lamina corresponding to Layer V in the areas already described can be made out in this region.

Layer VI. The line of demarcation between this and the superadjacent stratum is very indistinct. The polymorphic layer is here very thick, being thicker in comparison to the total depth of the cortex here than is the case in any other region except in Area 5. Large polymorphic cells—27 by 36 microns on the average—occur singly and in groups at different levels throughout this stratum.

Area 5. Cortex from the region of junction of thalamus and cerebrum. Average thickness 6.6. to 7 mm. (fig. 9).

Layer I. The zonal layer is well marked and shows a peculiar coarse reticulated appearance due to the presence here of great numbers of large thalamic projection fibers. At the periphery of this stratum there is a layer of small embryonic or neuroglial elements.

Layer II. The line of demarcation between this layer and the preceding is very indefinite. Numerous large and medium sized polymorphic elements, together with small embryonal cells make up this stratum. The embryonic elements, however, by no means predominate there as is the case in this stratum elsewhere in the cortex.

No lamina corresponding to Layer III in other regions can be distinguished here.

Layer IV. This stratum is indistinctly marked off from the layers above and below it. It is a thick lamina whose elements show a decided tendency to become progressively smaller in size from without inward.

There is no lamina corresponding to Layer V as described over the anterior lobe.

Layer VI. This is a very thick stratum and is poorly marked off from Layer IV. The elements are occasionally arranged in groups of irregular size in which the cells are more closely packed than in the intervening spaces. At various levels in this stratum, but more especially in the middle third, there are found certain very large giant cells occurring either singly or in groups of two or three. Many of these cells are as large as 36 by 54 microns but the average size is about 34 by 50 microns.

Most of these giant cells are of quite normal pyramidal type but not a few are of irregular polymorphic shapes. The nucleus is large, vesicular

and usually centrally placed, and shows as a rule a well developed eosinophile karyosome. In the cytoplasm Nissl bodies are present, but show as distinct and sharply defined granules only in some cases; in others the tigroid substance is very variable in its appearance. In all cases there is a well defined circumnuclear zone practically free from Nissl substance. In a few cases the nucleus is eccentric and irregular in shape.

In Weigert stained sections of these areas, the following distribution of medullated tissue was noted: Area 5, many medullated fibers in both zonal layer and medulla, no radial fibers; Area 4, a few medullated fibers in the zonal layer and numerous medullated fibers in the medullary center; Area 3, occasional medullated fibers in the zonal layer and a few present in the medulla; Area 2, no medullated fibers in the zonal layer and a very few in the medullary center; Area 1, no medullated fibers anywhere.

DISCUSSION

Cortical lamination

The cell lamination in this case shows a number of points of resemblance to that obtaining in the normal cortex. Thus, there is everywhere to be distinguished a zonal or outer fiber lamina and the cortex in all those regions not subject to marked modification by the entering thalamic fibers may be divided into an outer and an inner cell stratum by the lamina described as Layer III. This division, then, would correspond to that obtaining in the normal cortex where, as Bolton and Moyes (3) have shown, there is a very early splitting of the primary cortex into two layers by the formation of an inner fiber lamina.

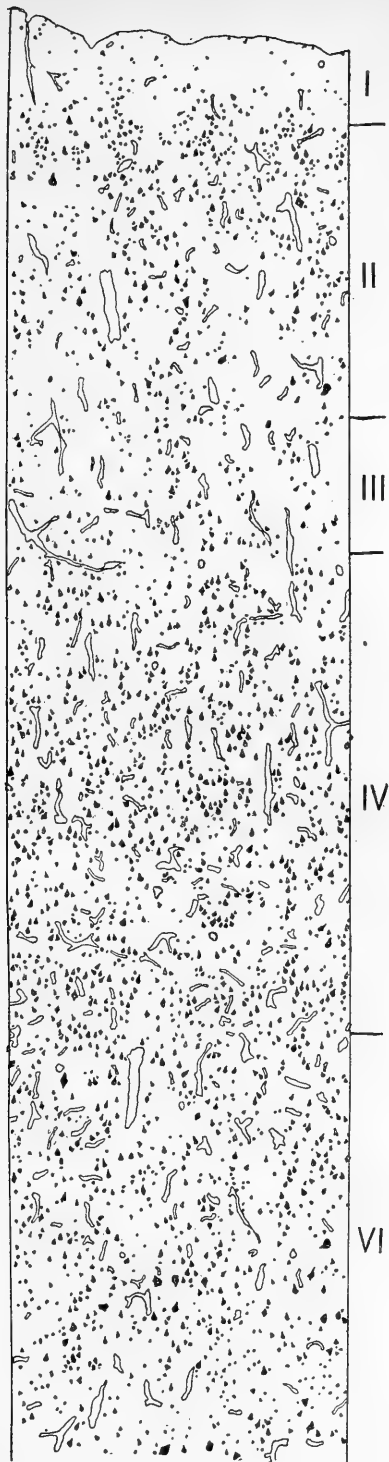
However, the lamination also shows several quite distinctive features not at all comparable to the normal. The stratum

Fig. 8¹ Section through cortex of posterior pole of the right posterior lobe. Area 4, region indicated in figure 2. $\times 65$.

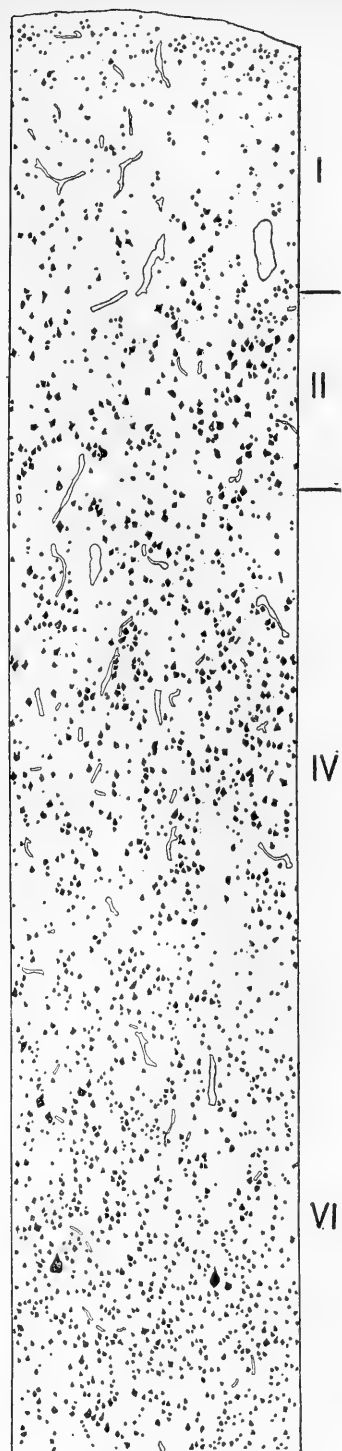
Fig. 9. Section through the cortex at the region of junction of the thalamus and cerebrum. Area 5, position indicated in figure 3. $\times 65$.

¹ In figures 8 and 9, certain cortical layers, which are described in figures 4 to 7, are not represented or numbered. It is not the intention, however, to imply that the laminae bearing the same numbers throughout these figures are necessarily altogether homologous. This point is made clear in the text.

8



9



described in the normal cortex as the inner fiber lamina has been shown to correspond to the inner line of Baillarger in the adult (3). This means that the greatest amount of cortical differentiation in man occurs normally in the outer cell lamina.

If, then, the stratum described as Layer III correspond to the inner fiber lamina of Bolton and Moyes, it will readily be seen that practically all of the cortical differentiation has taken place below this level in this case except in those areas markedly modified by the presence of projection fibers in the zonal layer. Disregarding these areas then, this cortex has conformed to the general embryological rule and has begun its development from within outwards. Beyond this the condition here seems to present an exception to any normal—'usual' might be a better term—cortical lamination. The inner cell lamina has become modified in an atypical fashion to form three fairly distinct layers while the outer cell lamina has apparently suffered involution.

It is also to be noted that any large pyramidal elements which simulate in form the cell bodies of efferent projection neurones are placed, not in relation to the homologue of the inner fiber lamina, where normally these cells are situated, but at much deeper levels in the much modified inner cell lamina.

Relation of cortex to afferent projection fibers

The atypical projection fibers of thalamic origin on gaining the cortex are not distributed equally to all areas. It thus happens that the cortex may be divided into two major regions: (1) a region in which thalamic fibers end, and (2) one destitute of such fibers. Area 1, described above, may be taken as typical of the histological formation characteristic of the cortex in those regions devoid of thalamic projection fibers.

Furthermore, the region supplied by thalamic fibers may be also subdivided according to the method of distribution of these into (a) a region in which the majority of the thalamic fibers enter by way of the outer or zonal layer, and (b) an area of greater extent containing fewer projection fibers which enter the cortex mainly by way of the medullary center. The histological arrange-

ment of the cortex in the latter region is illustrated in the preceding description by Areas 2 and 3, while that of the former which shows greatest variation from the general cortical pattern, is well shown in Areas 4 and 5.

Projection fibers entering mainly via zonal layer

Let us first consider the effect of the entering thalamic fibers when distributed mainly by way of the outer or zonal layer of the cortex. This is the case especially in Area 5, at the region of junction of the thalamus and cerebrum. The second layer of the cortex, which is, elsewhere, for the most part, made up of poorly differentiated pyramidal, polymorphic and embryonal cells, shows here a fewer number of elements on the whole and of these the greater number are quite large well differentiated pyramidal or polymorphic cells. It is of significance to note that the average size of the elements decreases while their number increases in passing more deeply into the cortex in this region. The various cortical laminae are much disturbed and difficult to make out. Certain very large giant cells occur in the deepest stratum and it is to be noted that this stratum bears the same relation to the projection fibers in the medullary layer as the second layer of the cortex bears to these fibers in the zonal layer.

In Area 4, large polymorphic cells are present in the second layer of the cortex in considerable numbers, though this condition is not so marked as in Area 5. A considerably smaller number of thalamic fibers are coursing in the zonal layer in Area 4. The cortical lamination here also shows great variation from that obtaining over the greater part of this cortex. As in Area 5, the polymorphic layer, which is also reached by thalamic fibers via the medullary center, here shows many well developed large cells, though none of these attain the size of the giant cells in the former area.

The thalamic fibers entering by way of the zonal layer are able to come into immediate contact with the dendritic processes of the elements of the second layer of the cortex. The effect is such as to cause elements which normally show least signs of spe-

cialisation at birth, that is, the outer cell layer of the cortex, to develop to a marked degree and become more highly differentiated than most of the elements at deeper levels.

The deepest stratum of the cortex in this area is also reached by thalamic fibers by way of the medullary center. This is the path along which these fibers pass under normal circumstances. In the deep cell strata there also occur very numerous large and medium sized well differentiated cells, and in addition in Area 5, giant cells are to be found here. The area of least differentiation occupies an intermediate position between the zonal and the polymorphic layers, and is the level least accessible to thalamic fibers.

Projection fibers entering mainly via medullary center

Passing on to the consideration of those areas reached by thalamic fibers mainly by way of the medullary center, it will be noted that the cell lamination approaches more closely the generalized type already described.

In Area 3, from the central portion of the base of the left posterior lobe, there are still certain thalamic fibers present in the zonal layer. As has been shown, irregular, quite large, multipolar elements are of frequent occurrence here either in the zonal layer or at irregular intervals throughout the second stratum. Layer III is here recognized for the first time as a lamina sparsely beset with small cells and dividing the cortex into an upper and a lower cell stratum. In the deeper cell strata the elements show quite an advanced state of differentiation and numerous medium and large pyramidal and polymorphic cells are found here. These deep strata are in closest relation to the thalamic fibers coursing in the medullary center and are evidently stimulated by this contiguity.

In Area 2, practically no thalamic fibers course in the zonal layer, yet here and there in the outer part of Layer II medium sized multipolar elements are to be found. The cell lamination in this region is more distinct than in Area 3 and the line of demarcation between Layers I and II is quite sharp. Layer III is also

distinct. The occurrence of well differentiated elements superficial to the latter layer is the exception rather than the rule over this area. In the deeper strata of the inner cell layer there occur either singly or in groups, numerous giant polymorphic or pyramidal cells. The smaller polymorphic elements at this level show a greater degree of differentiation than those at higher levels. Practically all the thalamic fibers reaching this area of the cortex course in the medullary center and enter the deep layers of the cortex first with the result noted.

If the hypothesis put forward in an earlier paper (2) to account for the form of the forebrain vesicle, in this case be carried to a logical conclusion in detail, it will be noted that the area bordering upon the divergent limbs of the Y-shaped median furrow would correspond in general to the Rolandic area in the normal cortex. I mention this only on account of the curious coincidence of the presence of a well marked series of giant cells in the deeper cell strata in this area and regard it as a coincidence only.

Regions destitute of thalamic fibers

Areas 1 A and 1 B represent the type of lamination characteristic of those areas quite destitute of the influence of thalamic projection fibers. These two areas, though wide apart, show essentially similar types of lamination. The only real difference in their structure lies in the fact that, as Area 1 B is nearer the cortical limbus, the cortex as a whole is much the thinner of the two. It is over these areas that cell differentiation is found to be least in evidence, while the total number of elements is considerably greater in a given section in these areas than in those reached by thalamic fibers in abundance.

It is to be noted that despite the close crowding of cells alluded to above, the total thickness of the cortex in Area 1 A is greater than that found in any other area except those in the immediate neighborhood of entering thalamic fibers. In the latter areas the great thickness is at least in part due to the scattered arrangement of the cells resulting from the influence of the presence of these atypical fibers. Thus I am led to conclude that, whatever

the condition of cellular activity may be in other areas of the cortex, this portion certainly shows evidence of hyperplasia. This hyperplasia is associated with diminished specialisation of elements as one might expect, yet it is significant to note that the increase in cell content has been conducted in an orderly fashion resulting in a special type of lamination characteristic of this case.

Trophic activities of afferent fibers

Roux has pointed out (5) that the development of an active tissue (that is, muscular, nervous or glandular) may be divided into two phases, one which he terms 'self differentiation' in which development goes on without regard to any functional connections, and a second stage termed 'dependent differentiation' where further normal development is not possible unless the tissue functionates.

Bechterew (1) has shown that the trophic activity of nervous tissue upon nervous tissue, if the supply of nutritive material continue to be normal, depends upon the functional continuity of the neurone systems.

Thus in cortical development, normal differentiation and evolution will cease after the period of 'self differentiation' has passed unless there is functional connection established with lower nervous centers. The end of this period of 'self differentiation' in cortical development is marked in all probability by the first entrance of afferent projection fibers. Full development is reached, as v. Bechterew points out, only when the reflex arc is completed, that is when the efferent projection fiber has established its peripheral connections. These generalities of course apply only to projection centers, but as association areas are primarily dependent upon projection centers in their development, it is not necessary to consider them in this connection.

There is thus a critical point, as it were, beyond which, in the absence of functional connections, any attempts towards differentiation will take place along quite unusual lines.

In this case afferent projection fibers reached certain areas of the cortex in an abnormal way and came in contact with neurones in the outer cell strata. The effect of this contact was to start

these neurones on the second phase of their development, namely, 'dependent differentiation,' in a quite atypical manner. In other words, the primary action of the afferent fibers on entering the cortex is of a trophic nature. No doubt under normal circumstances this action is more or less selective in character but when, as in this case, these fibers are abnormally situated, their effect is still evidenced by a growth excitation in the area of their distribution.

Thus in Areas 4 and 5 the fundamental pattern laid down during the stage of 'self differentiation' has been much modified by the atypical relations of these areas to the afferent thalamic fibers. This disturbance is so extensive that the division into an outer and an inner cell lamina is to be made out only with difficulty.

Areas 2 and 3 serve as excellent intermediate stages illustrating this. Here fewer afferent fibers course in an abnormal fashion, and Layer III which indicates the line of demarcation between the outer and inner cell strata is quite evident.

Results of absence of afferent trophic action

If, then, the primary action of developing afferent projection fibers be trophic, there are certain areas in this cortex which lack this trophic control. These areas should have passed the normal stage of 'self differentiation' and, lacking trophic connections, will have entered on an abnormal stage of development.

It is to be noted here that the blood supply, as evidenced by the size and number of blood vessels, is rich and all areas are apparently equally well supplied.

This abnormal development of the cortex lacking the usual trophic control has resulted in the appearance of three characteristic variations from the normal: first, the atypical development of the inner cell stratum into three layers; second, the marked lack of differentiation of the cells at all levels; and third, the even more marked increase in total number per given section of cell elements and the consequent increased thickness of the cortex over this area.

The result, then, of the aberrant afferent trophic action upon this developing cortex has been to cause a marked hyperplasia,

more especially of the inner cell stratum, with the resulting formation of atypical laminae, together with a greatly increased total thickness of a cortex made up of closely crowded poorly differentiated cells. This latter condition is evidently associated with the hyperplasia which is, in general, opposed to specialisation.

It is of interest to note that in tissues of mesodermal origin lacking trophic control, somewhat similar phenomena have been shown to occur. Cehanović (4) has demonstrated that the removal of trophic control in the vascular system, is accompanied, more especially in arteries, by a marked hypertrophy and hyperplasia of muscular and connective tissue elements in the media and also of the elements of the intima.

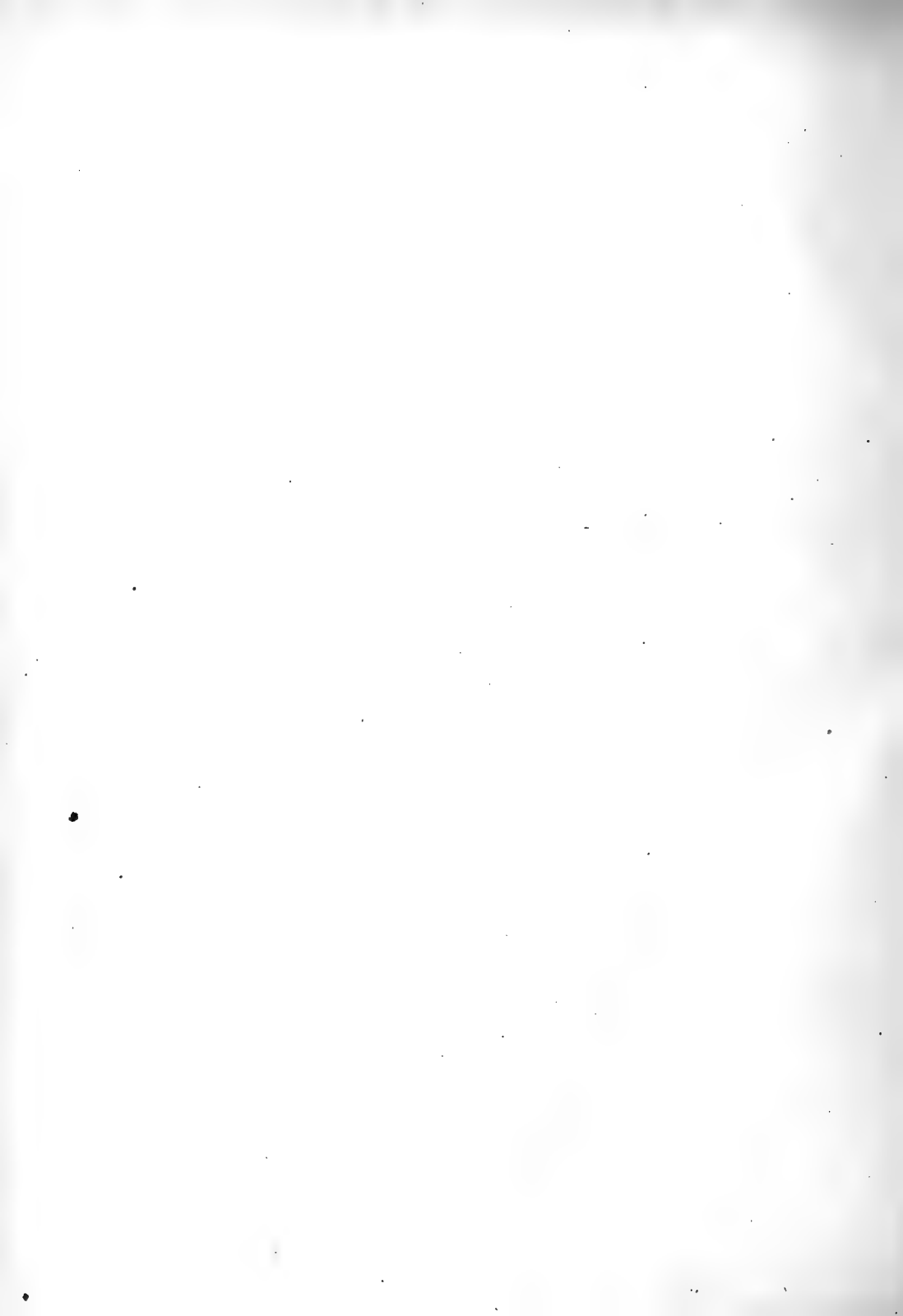
SUMMARY

The points illustrated by the condition of cortical development in this case may be summarized as follows:

1. When afferent projection fibers are present in abnormal locations, their presence may result in atypical growth and differentiation of neurones which are usually characterised by the regularity of their form and their small size.
2. The presence of these afferent fibers apparently provides the necessary stimulus toward differentiation required by the large efferent neurones. Furthermore the elaboration and differentiation of the primary outer cell lamina of the cortex is dependent upon the establishment of the normal functional connections of these two units.
3. When the normal trophic stimulus provided by the afferent projection fibers in the cortex is lacking, there results an atypical cortical development which may be compared to the hyperplasia occurring in many tissues of mesodermal origin in the absence of their trophic control.
4. The cell lamination resulting from the disturbance of afferent connections is atypical and characteristic of the case. It is thus fruitless to attempt to identify accurately by means of the lamination pattern, any cortical area in a case such as this with the histologically different areas in the normal cortex.

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THE MORPHOLOGY OF THE SEPTUM, HIPPOCAMPUS, AND PALLIAL COMMISSURES IN REPLILES AND MAMMALS¹

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NINETY-THREE FIGURES

In the mammalian brain the hippocampus extends from the base of the olfactory peduncle over the corpus callosum and bends down into the temporal lobe. Over the corpus callosum there is a well developed hippocampus in monotremes and marsupials, while in higher mammals it is reduced to a slender vestige consisting of the stria longitudinalis and indusium. The telencephalic commissures in monotremes and marsupials form two transverse bundles in the rostral wall of the third ventricle. We owe our knowledge of the history of the pallial commissures in mammals chiefly to the work of Elliot Smith. This author states that these commissures are both contained in the lamina terminalis. The upper (dorsal) commissure represents the commissure hippocampi or psalterium; the lower (ventral) contains the commissura anterior and the fibers which serve the functions of the corpus callosum. In mammals as the general pallium grows in extent there is a corresponding increase in the number of corpus callosum fibers. These fibers are transferred from the lower to the upper bundle in the lamina terminalis, in which corpus callosum and psalterium then lie side by side. As the pallium grows the corpus callosum becomes larger, rises up and bends on itself until it finally forms the great arched structure which we know in higher mammals and man.

During all this process two changes have taken place in the lamina terminalis. First, it was invaded by cells from the neighboring medial portion of the olfactory lobe so that the paired

¹ Neurological studies, University of Minnesota, no. 18.

medial olfactory nuclei came to be connected by a median mass through which the commissures crossed from side to side. The median mass was called, by Elliot Smith the commissure bed and the whole body, including the paired gray masses, was called the paraterminal body. Second, the enlargement and arching up of the corpus callosum caused the stretching of the lamina terminalis and the commissure bed. The space within the concavity of the callosal arch was filled by a part of the paraterminal body. This part of the medial wall is thick in lower forms but the great arching of the corpus callosum in higher mammals has stretched it out into the thin septum pellucidum.

² This very clear conception of the commissures and their relationships has been generally accepted and has been followed by the writer. Elliot Smith and others have carried this conception into the study of reptiles and have based upon it some homologies in the brains of amphibians and fishes. The writer has approached all questions of brain morphology from a different standpoint. He has directed his attention always to the more primitive forms, making the attempt first to see the structure and relationships existing in those brains, with the hope that thereafter it would be possible to see with certainty how the specialized brains of higher forms have been developed from the lower. The method heretofore followed by most workers has been to study man and mammals first, as the animals in which we are directly interested, and then to apply the results to lower vertebrates either as a matter of curiosity or as a means of securing corroborative evidence of their accuracy. The genetic method is more tedious but if followed with caution and fidelity to the actual facts it should lead to permanent results. Caution is necessary in the forms chosen for study and the significance accorded to the facts discovered. If we are to use lower forms to throw light upon the human brain, those forms must be chosen for study which are at once the least specialized and most nearly allied to the ancestors of mammals and man. In studying such brains attention must be given to the habits of the animals and the consequent degree of development of each system of nerve centers.

The work of the writer has shown that the telencephalon of cyclostomes is truly primitive as compared with that of other fishes. It is believed that the more primitive selachians are the nearest existing allies to the common ancestors of fishes, amphibians and reptiles. Among the reptiles those which are regarded by paleontologists as nearest the line of mammalian descent are the chelonia.

The writer's reasoning has been that an understanding of the morphology of the mammalian and human brain must rest on a clear knowledge of the steps in its evolution and of the forces (habits, mechanical factors, etc.) which have been concerned in that evolution. For this purpose we must be able to give a consistent account of the evolution of the brain through the chief groups of vertebrates which stand nearest the line of descent of higher mammals: the cyclostomes, selachians, dipnoi, chelonia, monotremes, marsupials, and certain mammals. With this in view the writer, having studied the telencephalon in cyclostomes and selachians, wishes to present here some contributions upon reptiles and mammals. Further studies of dipnoi, as affording a bridge between selachians and reptiles, are greatly to be desired.

The reason for the above statement of the writer's method of approach to this problem is that the facts thus far observed in the telencephalon of primitive vertebrates do not agree with the accepted views regarding the mammalian brain. The difficulties have to do chiefly with the relations of the paraterminal body, lamina terminalis, commissures and hippocampus. In selachians the pallial commissures are imbedded in a massive roof (called by the writer the *primordium hippocampi*) and cross through the lamina *supraneuroporica*, wholly independent of the lamina terminalis and the paraterminal body. The fiber connections of the body called *primordium hippocampi* are so characteristic and the line of demarcation between it and the paraterminal body is so clear, that there can be no question of the individuality of the *primordium hippocampi* in selachians and cyclostomes. The only doubt which has arisen in the use of this term has been whether the body in question may not give

rise to something more than the hippocampal formation in the mammalian brain (Johnston '10 c, p. 149). With the study of the brains of a number of reptiles and mammals, the conviction has gradually forced itself on the writer that the prevailing ideas regarding the morphology of the medial wall of the telencephalon in mammals must be modified in view of the fundamental relations in lower vertebrates. The evidence for this can best be presented by careful comparison of the chief structures in this region in lower and higher vertebrates.

For the sake of clearness it must be understood that the term *primordium hippocampi* is used in the sense in which the writer has employed it in his previous papers. The fitness of this and other terms is discussed in a section at the end of this paper.

THE ROOF-PLATE IN THE TELENCEPHALON

The anterior end of the brain floor is occupied by the optic chiasma ('09 b). The roof-plate begins at the preoptic recess and extends in a curve around the topographic rostral end of the brain and caudad into the roof of the diencephalon ('10 c). The *velum transversum*, always present in vertebrates at least in embryos, marks the boundary between diencephalon and telencephalon in the roof ('09 b). The telencephalic roof-plate consists of three portions, the *lamina terminalis*, *lamina supraneuroporica* and *tela chorioidea* ('11 b, p. 491). The *lamina terminalis* is formed by the fusion of the lips of the primitive neuropore and is bounded above by the neuroporic recess which marks the point of latest connection of the neural tube with the ectoderm. The *lamina supraneuroporica* is thickened by commissural fibers in cyclostomes and by gray matter and fibers in selachians, and even in ganoids and bony fishes where it usually contains no commissures it is thickened and is histologically different from the *tela chorioidea* ('11 b). From the *tela chorioidea* just rostral to the *velum transversum* arises the *paraphysis*. The attachment of the *tela chorioidea* to the dorsal border of the wall of the telencephalon medium is known as the *taenia fornicis* ('11 a, p. 6). The *taeniae* of the two sides converge rostrally to meet at the point of junction of the *tela chorioidea*

with the lamina supraneuroporica in the mid-line. This point has been labelled *m* in numerous figures in preceding papers. Since there is an early differentiation between the tela chorioidea and the lamina supraneuroporica in the embryo, this point is always easily recognizable in both embryos and adults of all vertebrates. It is one of the fundamental landmarks in the telencephalon. Its position has been noted by Elliot Smith ('97 b, p. 52, fig. 23; '97 c, p. 235) and is indicated in figures 4, 5, 6, 7, 9, 35, 45, 55, 64 of this paper. This point may be named *margo posterior pallii*.

To complete the identification of lamina terminalis and lamina supraneuroporica in reptiles and mammals it is necessary only to determine the position of the recessus neuroporicus.

The neuroporic recess is always situated rostral or dorsal to the anterior commissure. Dorsal to the recess in cyclostomes and selachians is the lamina supraneuroporica with its commissures and gray matter as already mentioned. In selachians there is found an external neuroporic recess ('11 a) in the form of a deep pit or a slender canal, through which blood vessels enter the brain near the upper border of the lamina terminalis.

In all the embryos of reptiles and mammals which the writer has examined there is found a ventricular recess rostral to the anterior commissure, and above the recess is a thick lamina which in advanced embryos contains a pallial commissure. These relations were shown in a previous paper ('10 c, figs. 17, 18, 19, 20). It will be seen at once upon comparison with selachian embryos that the relations are essentially the same. This recess is not preserved in the adults of all reptiles and mammals, but its position is readily determined, since it must lie between the anterior and the pallial commissures. The recess which is found in this position is the recessus triangularis of Schwalbe ('81). Its significance will be discussed in the following paragraphs.

The location of the recessus neuroporicus is of critical importance. Most authors who have recognized a neuroporic recess in mammals have identified it with the angulus terminalis of His and the recessus superior, the relations of which are so clearly figured by Elliot Smith in monotreme, marsupial and mammalian brains.

His in his *Allgemeine Morphologie* in 1892 (p. 349, 352) defined the lamina terminalis as the frontal portion of the frontal seam of closure. Below, the lamina terminalis ends in the recessus opticus (rec. praeopticus) and is followed by a basal portion of the frontal seam containing the optic chiasma. The upper border of the lamina terminalis in human embryos is marked by an inward fold (i.e., the velum transversum) in early stages and later by the beginning of the choroid plexus. Within the lamina terminalis as thus defined (cf. p. 349 and 352) in many animals lies the neuropore.

Von Kupffer ('93) described the neuropore in the sturgeon under the name of lobus olfactorius impar. Von Kupffer's material was unfortunate because the eversion of the pallial part of the telencephalon in ganoids leaves the recessus neuroporicus apparently bounded dorsally by a non-nervous membrane only. Comparison of v. Kupffer's figure 18 with the writer's figure of this region in the adult sturgeon brain ('11 b, fig. 52) will show that there intervenes between the locus of the recessus neuroporicus and the plexus chorioideus a membrane which is distinctly thicker than the choroid plexus and differs from it histologically. v. Kupffer ('94) also described the recessus neuroporicus in *Petromyzon* and it has since been shown by Sterzi ('07) that this lies below the dorsal telencephalic commissure. Upon direct comparison of the adult human brain with that of the sturgeon embryo, v. Kupffer believed (p. 39) the recessus triangularis of Schwalbe ('81) to be the homologue of his lobus olfactorius impar.

In the comments made by His ('93) upon v. Kupffer's work, he identified v. Kupffer's lobus olfactorius impar with the angulus terminalis of His. This angulus terminalis is the meeting point of the tela chorioidea with the dorsal border of the lamina terminalis as defined in 1892. This does not agree with v. Kupffer's view cited above, nor does it agree with His's own statement in 1892 that the neuropore lies within the lamina terminalis.

Burckhardt ('94 b) identified v. Kupffer's lobus olfactorius impar with a recess in reptiles situated at the point of junction of

the choroïd plexus with the lamina containing the dorsal commissure. To this recess Burckhardt gave the name recessus neuroporicus and to the ependymal membrane which stretches from this recess to the paraphysis he gave the name lamina supraneuroporica. Burckhardt ('94 c) then compared this ependymal lamina supraneuroporica with the tela chorioidea of various fishes, implying that the neuroporic recess was located at the point of attachment of the tela to the massive roof in front. In all this Burckhardt was in agreement with His 1893. In his last work, however, Burckhardt ('07) clearly described the neuroporic recess in Scymnus and shows that in selachians the lamina supraneuroporica is a true nervous structure. It is in this sense that the term lamina supraneuroporica must be used and not in the sense in which Burckhardt first used it.

In the meantime Elliot Smith ('96 a, '99) following Burckhardt, identified the recessus superior of mammalian brains with the angulus terminalis of His and with the recessus neuroporicus. There can be no doubt that the recessus superior is the same as the angulus terminalis (figs. 8, 9, *m*) and that it corresponds to the recess which Burckhardt called neuroporic recess in reptiles. In both classes this recess lies just over the dorsal telencephalic commissure and is bounded above by the tela chorioidea, as clearly figured by Elliot Smith. That it does not correspond to what is known to be the neuroporic recess of selachians is obvious, since in the latter case the neuroporic recess is separated from the tela chorioidea by a thickened lamina containing the dorsal commissures.

The chief contribution by v. Kupffer in this connection was his pointing out the importance of the neuroporic recess as a landmark in forebrain morphology. His in 1892 presented a clear conception of the neural tube as closed rostrally by the lamina terminalis within whose extent the neuropore appeared in some animals. In 1893 he inadvertently abandoned this definition by placing the neuropore at the dorsal border of the lamina terminalis where the latter meets the choroid plexus. Since then some authors have defined the lamina terminalis as *the membrane formed by the closing of the neuropore and bounded*

dorsally by the neuroporic recess. Other authors have defined the lamina terminalis as *the closing membrane which is bounded above by the choroid-plexus.* Both groups of workers believe that they are following His. The result has been the greatest confusion. In some cases even the tela chorioidea has been included in the lamina terminalis. The writer holds the view that the lamina terminalis is coextensive with the primitive neuropore and is bounded above by the neuroporic recess, but insists that the locus of this recess shall be accurately determined. It will appear below that His was in error in locating the neuroporic recess at his *angulus terminalis*.

Conclusive evidence as to the location of the neuroporic recess in reptiles and mammals is to be obtained only by following that recess from the open neuropore through successive stages of development until its relation to the telencephalic commissures is established. The accompanying figures 1 to 6 show the contour of the dorsal seam of the forebrain in embryos of *Chelydra serpentina* from the stage of the open neuropore to a stage possessing a carapace 8 mm. in length. For the opportunity to study this material the writer is indebted to Dr. C. E. Johnson. The locus of the neuroporic recess is entirely clear throughout these stages. In the latest stage drawn (fig. 6) there appears between the preoptic recess below and the beginning of the tela chorioidea above (*m*) a somewhat S-shaped thickening of the roof-plate. In the lower part of this thickening the fibers of the anterior commissure are present. Above this the neuroporic recess appears as a ventricular pit. The upper part of the thickening is the lamina supraneuroporica.

The same relations are seen in figure 7 which shows the outline of the median section of the brain of a slightly more advanced embryo of *Emys lutaria*. For the opportunity of studying this embryo I am indebted to Dr. G. Carl Huber. In this the recessus neuroporicus separates widely the lamina supraneuroporica from the thickening in the lamina terminalis which contains the anterior commissure. The lighter outlines numbered 1, 2, 3, 4, are the outlines of successive parasagittal sections of the medial wall of the right hemisphere. The contours 1 and

2 show that an oblique ridge runs upward and forward from the lamina supraneuroporica into the medial wall of the hemisphere. In the brain of the adult turtle (fig. 9) this ridge is occupied by the hippocampal commissure. The recessus neuroporicus is evident in the adult upon the inner surface of the transverse ridge which is occupied by the anterior and the hippocampal commissures (fig. 9). In *Chelydra serpentina* the two commissures form a ridge projecting farther into the ventricle and the neuroporic recess is nearly obliterated.

Elliot Smith's ('03) figures and clear description of *Hydrosaurus* show that a deep recess (his recessus inferior) exists between the anterior and hippocampal commissures in this Monitor. Professor Smith points out that this recess corresponds to the recessus triangularis of Schwalbe in mammalian brains.

In his work on the pineal region of *Sphenodon*, Dendy ('10) shows in text-figure 1 a median section which agrees very well with my latest stage of *Chelydra serpentina*. In agreement with Burckhardt's earlier work he applies the name lamina supraneuroporica to the tela chorioidea, while to the thick lamina to which the tela is attached he gives the name cerebral hemisphere. Although of course the hemisphere can not really be seen in a median section, the application of this name by Dendy serves to show that the structure which the writer has called lamina supraneuroporica is a nervous bridge connecting the two hemispheres in *Sphenodon* as in the turtles.

The same conformation in the region of the anterior and hippocampal commissures in a late embryo of *Anguis fragilis* is shown in v. Kupffer's figure 248 in his article in Hertwig's *Handbuch*.

From his own observations on turtles and from the comparison of the results of other workers, the writer is convinced that the neuroporic recess in reptiles is located immediately above the anterior commissure and that the hippocampal commissure crosses the middle line in a lamina supraneuroporica which serves as a massive bridge between the medial walls of the two hemispheres. In these relations the reptiles agree strictly with the selachians.

We have fairly clear and complete evidence as to the location of the neuroporic recess in mammals. In a previous communication ('09 b) the writer identified as this recess a pit located in the pig of 15 mm. and larger just above the anterior commissure. Figure 39 of that paper may be consulted in this connection. The development of the pig has since been carefully reviewed and the writer is convinced of the correctness of this view. The pit in question is the embryonic representative of the recessus triangularis of Schwalbe and lies over the anterior commissure and between the fornix columns. In a later communication ('10 c) were given median sections of rabbit and cat embryos showing the same recess between the anterior and the pallial commissures. Sheep embryos show the same relations. Figures 8 and 24 show the neuroporic recess and lamina supraneuroporica in a human embryo of 37 mm., for the opportunity to study which I am indebted to Dr. G. Carl Huber.

Neumayer ('99) figures early stages of rabbit and sheep embryos in which he clearly identifies the neuroporic recess (under the name of lobus olfactorius impar) and shows that it comes to lie between the anterior and pallial commissures. Several of his figures are reproduced in Ziehen's article on the morphogenesis of the central nervous system of mammals in Hertwig's Handbuch. The pit which the writer identified as neuroporic recess in cat embryos was figured by Martin ('93) who showed that the so-called lamina terminalis grows thinner in later stages at the location of this pit. It is well known that the recessus triangularis (rec. inferior of Elliot Smith) exists in this same position in adult monotremes, marsupials and many mammals. Werkman ('13) in his recent work on the development of the commissures in lower mammals shows the neuroporic recess in embryos of the mole and the bat (figs. 5, 8, 13, 15, 17, 18, *c.ep.*).

It is proposed that hereafter the name recessus neuroporicus be adopted for this pit in the brains of all vertebrates, instead of recessus triangularis (Schwalbe) or recessus inferior (Elliot Smith). The recessus neuroporicus may be defined as a pit existing in the embryos or adults of all vertebrates in the topographic rostral wall of the third ventricle, marking the point at which the dorsal seam of closure of the neural tube last separated

form the ectoderm. It lies always topographically dorsal (or rostral) to the anterior commissure and in all forms which possess a true fornix and pallial commissures it lies beneath the pallial commissures and between the columns of the fornix.

The lamina terminalis is the anterior portion of the roof-plate and is formed by the fusion of the lips of the anterior neuropore. It extends from the anterior border of the chiasma ridge to the neuroporic recess and is traversed by the anterior commissure. The lamina supraneuroporica is a thickened portion of the roof-plate extending dorso-caudad from the neuroporic recess and containing pallial commissures in cyclostomes, selachians, some ganoids and teleosts, dipnoi, reptiles and mammals. The tela chorioidea telencephali is the anterior part of the membranous roof of the third ventricle, attached rostrad to the lamina supraneuroporica, containing within its extent the paraphysis, and separated from the tela chorioidea diencephali by the velum transversum. The evidence on which these definitions rest has been examined by the writer personally in all classes of vertebrates except the Dipnoi. For information regarding the position of the pallial commissures in Dipnoi I am indebted to Prof. G. Elliot Smith who states in a letter that the pallial commissure in *Lepidosiren* is disposed as in reptiles.

GROSS RELATIONS IN THE MEDIAL WALL OF THE HEMISPHERE

In evaginated brains such as those of the selachians, amphibians, reptiles and mammals the hemisphere projects rostrad beyond the lamina terminalis and possesses a medial wall. Between the apposed medial walls of the two hemispheres is the great sagittal fissure which is closed caudally by the lamina terminalis and lamina supraneuroporica. The lower part of the medial wall (olfactory lobe) undergoes a secondary fusion in most selachians. In reptiles and mammals there is only a thickening of the lamina terminalis and of the lamina supraneuroporica related to the anterior and pallial commissures. In selachians these thickenings and the secondary fusions become so extensive that the external neuroporic recess is reduced to a slender canal. Along the line of this canal the medial wall presents a cell-free

zone reaching from the vicinity of the neuroporic recess around the rostral end of the hemisphere into the lateral wall. This cell-free area which the writer has called the *zona limitans hippocampi*, marks the limit between secondary olfactory centers below and the olfacto-gustatory correlating center above, the *primordium hippocampi*.

In the comparison of the telencephalon of selachians with that of reptiles and mammals the identification of this *zona limitans* would be of the greatest convenience. It may be said at once that this boundary line is not always easy to recognize. A cell-free zone occurring in the medial wall of the hemisphere is not necessarily homologous with the *zona limitans hippocampi* of selachians. The line of demarcation between any two functionally differentiated nuclei often presents itself in the form of a cell-free zone. It is therefore necessary to examine the relations of the centers in question. In all vertebrates the secondary olfactory centers receive olfactory tract fibers and send fibers to the hypothalamus and to the nucleus habenulae. In selachians, if we confine our attention to the medial wall of the hemisphere, three kinds of fibers pass across the *zona limitans* between the medial olfactory nucleus and the hippocampal *primordium*: (a) olfactory tract fibers, (b) fibers arising from the cells of the medial olfactory nucleus and *tuberculum olfactorium*, known as the *tractus olfacto-corticalis septi*, and (c) the *fornix columns* which descend from the hippocampal *primordium* to pass caudad. In selachians the *primordium hippocampi* presents no arrangement of cells into layers. The medial olfactory nucleus presents two poorly marked cell layers. The outer one is continuous below with the more compact and definite cortical layer of the *tuberculum olfactorium*. This nucleus is present in reptiles and mammals but has received little attention from previous authors. It is shown in Herrick's figures 46 and 66 where it is called *nucleus medianus septi*; but the nucleus so named in figure 43 is the *primordium hippocampi* and the nucleus so named in figure 47 is that part of the *paraterminal body* which surrounds the *recessus praeopticus*. The deeper nucleus forms a projection into the lateral ventricle and has been called *nucleus accumbens septi* (Kappers and Theunissen) *nucleus septi* (Unger)

and nucleus lateralis septi (Herrick). I shall hereafter speak of the medial olfactory area as the area parolfactoria and shall use for the two nuclei contained in it the names nucleus parolfactorius medialis and lateralis, respectively. The general features of the relations in the medial wall in selachians are shown in figure 10.

When the medial wall of a reptilian brain is compared with that of the selachian there seems at first sight no great difficulty in identifying the hippocampal formation and the area parolfactoria. The hippocampus has been identified with the medio-dorsal cortex by Meyer ('92), Elliot Smith ('96, '10), Levi ('04) and others. Below the rostral end of the hippocampal formation the area parolfactoria is thickened so as to form a ridge in the ventricle. This is the anterior end of the paraterminal body of Elliot Smith and according to the account given by that author ('03) this body becomes united with its fellow through the lamina terminalis, thus forming the bed of the anterior and hippocampal commissures. The paraterminal body also extends caudad in the medial wall of the hemisphere over the interven-tricular foramen. In certain forms this supraforaminal portion of the paraterminal body continues to the caudal pole where it again unites with its fellow (e.g., in *Sphenodon*) to form the bed of the commissura aberrans. In mammals it is stated that this paraterminal body surrounds and embeds the entire system of hemispherical commissures (c. anterior, c. hippocampi and corpus callosum). These interpretations are diagrammatically represented in figures 82 and 83.

The problem before us is to determine what parts of the medial wall of the hemisphere in reptiles and mammals have been derived from that definite morphological and physiological unit in selachians which the writer has called the primordium hippocampi, and what parts have been derived from the area parolfactoria. The examination which follows will show that the paraterminal body of Elliot Smith includes parts of both the area parolfactoria and the primordium hippocampi of selachians and that the bed of the pallial commissures is of different origin and has different morphological relations from the bed of the anterior commissure.

In figures 14 and 17 are shown two transverse sections of the telencephalon of the hinge turtle (*Cistudo carolina*), one near the olfactory peduncle and one near the interventricular foramen. In the section near the foramen the locus of the recessus neuroporicus is indicated by the position of the anterior commissure. If we compare in the turtle and the selachian that portion of the hemisphere which lies dorsal to the level of the neuroporic recess, we must recognize a differentiation of this region in the reptile's brain into two parts. The dorsal portion is the hippocampal formation and is bounded by the sulcus limitans of Elliot Smith ('03), *s.f.d.* in the figure. The remainder of the area would be regarded as a remnant of the primordium hippocampi of selachians in which differentiation of cortex has not taken place. Within this primordium are found the commissura hippocampi and the upper part of the fornix columns, as is the case in selachians. In the lateral ventricle a groove is seen at the level of the foramen which would be considered as the ventricular boundary of this primordium hippocampi. The body which we are thus hypothetically calling primordium hippocampi is of course the same that has been compared by Meyer ('92) and Unger ('06) with the septum pellucidum of mammals.

As the transverse sections are followed forward this thickening continues but the appearance of distinctness between it and the paraterminal body below gradually disappears. Deferring comment on sections in this intermediate area we may pass to the examination of figure 14. The section is taken behind the olfactory peduncle. The hippocampal formation is clearly marked in the medio-dorsal wall. The cortical layer stops suddenly and a small portion of the medial wall is composed of small cells without regular arrangement. This was called by Meyer part of the septum pellucidum. Below this is a thickening projecting into the lateral ventricle, apparently the same one as was seen near the foramen interventriculare. There can be no doubt of the interpretation of these three portions of the medial wall. The lowest is directly continuous with the tuberculum olfactorium below and receives olfactory tract fibers. It belongs to the area parolfactoria and paraterminal complex. The uppermost

is hippocampal cortex. The small body between is undifferentiated primordium hippocampi. The slight sulcus dorsal to it is the sulcus limitans hippocampi of Elliot Smith ('03). Its morphological significance will be discussed later. The deeper sulcus below corresponds to the sulcus limitans hippocampi as that term was used by the present writer ('11 a) for selachians. A cell-free zona limitans extends through the medial wall opposite to it.

The question at once arises, how can the same ridge or thickening in the medial wall be the medial olfactory nucleus at the rostral end of the brain and the primordium hippocampi at the foramen interventriculare, especially since a hippocampal primordium lies dorsal to this ridge at the rostral end of the brain? With the exception of Meyer and of Unger who distinguished between the septum pellucidum and the olfactory nucleus ("vorderes mediales ganglion," nucleus septi), previous authors have considered this whole region as belonging to the paraterminal body. An examination of the entire brain at once shows the error of this assumption. When the lateral ventricle of an adult turtle's brain is opened by removing the lateral wall, the ventricular surface of the medial wall shows the structures seen in figure 19. Rostrally the ventricle is constricted at the level of the olfactory peduncle by a transverse fissure dorsally and by a large longitudinal ridge in the ventro-medial wall. This ridge continues caudad in the lower part of the medial wall and is the medial olfactory nucleus or area parolfactoria seen in figure 14. Above this ridge is a groove in which under a hand lens there are to be distinguished two ventricular sulci corresponding to the two sulci on the medial surface above mentioned (fig. 14). Of these sulci the dorsal one continues caudad in nearly a straight line above the level of the interventricular foramen. The more ventral sulcus, about midway between the olfactory peduncle and the foramen, turns rather abruptly ventrad, forming a rounded caudal boundary to the olfactory ridge, and then bends caudad to reach the interventricular foramen (fig. 19). The body lying between these two grooves is the ridge seen in figure 17 and hypothetically identified above as the primordium hippo-

campi. It is continuous rostrally with the undoubted primordium hippocampi of figure 14 and is separated from the medial olfactory ridge (area parolfactoria) by a continuous ventricular sulcus. This sulcus has been overlooked by previous workers because they have depended upon the study of sections. After it has been seen in the dissected brain it can be traced in sections (figs. 11 to 17), but owing to its curve it comes to lie at one point so nearly in the plane of transverse sections (fig. 15) as to be very inconspicuous.

The parolfactory ridge above described has been figured by Unger ('06) in sections of the brain of Gecko under the name of nucleus septi, although he describes and figures it as a part of the area parolfactoria. Kappers and Theunissen ('08, fig. 19) call this the nucleus accumbens septi and name the sulcus which bounds it the fovea septo-striatica, considering the nucleus as a part of the striatum. Herrick ('10) cites Kappers and Theunissen but identifies the nucleus in *Lacerta* (fig. 43) with his nucleus lateralis septi which is in reality part of the primordium hippocampi. That is, he has overlooked the sulcus limitans hippocampi of the above description and has combined the area parolfactoria and the greater part of the primordium hippocampi under the name nucleus lateralis septi.

Upon the medial surface of the hemisphere of a turtle's brain, the outlines of the area under consideration can be made out almost equally well (fig. 20). The rostral portion of the lower half of the wall is occupied by an almost circular area which is continuous with the tuberculum olfactorium in the ventral wall. This clearly corresponds to the medial olfactory nucleus or area parolfactoria of the selachian brain. Dorsal to it are two sulci (cf. figs. 13, 14, 15 and 20). The lower one continues upon the ventral surface as the caudal boundary of the tuberculum olfactorium. The more dorsal sulcus extends from the olfactory sulcus back over the interventricular foramen. These two sulci correspond to the two above described in the ventricular surface of this wall. The more dorsal sulcus is the one called by Elliot Smith the sulcus limitans hippocampi because it forms the ventral boundary of undoubted hippocampal formation. The more

ventral sulcus corresponds to the sulcus limitans hippocampi described by the writer in selachians. The part of the medial wall between the two sulci is an undifferentiated portion or vestige of the primordium hippocampi of the selachian brain.

The primordium hippocampi thus outlined contains the commissura hippocampi and together with the hippocampal cortex recognized by previous workers, is the equivalent of the primordium hippocampi of selachians. Compare figure 85.

In Alligator mississippiensis the features described above are repeated so exactly that it is unnecessary to present separate drawings. There are differences in general form, and the area parolfactoria is relatively smaller than in the turtle.

The brains of various mammals, embryonic and adult, have been examined with reference to the gross relations of the primordium hippocampi and the area parolfactoria. In the opossum (*Didelphys virginiana*) these structures have essentially the same form as in the turtle. The chief difference is that the area parolfactoria is less prominent in the lateral ventricle and the sulcus limitans hippocampi is broader and more shallow. The medial wall as seen from the lateral ventricle is drawn in figure 21, which should be compared with figure 19. The medial surface of the same hemisphere is shown in figure 22. In this it will be seen that the only important difference from the turtle brain is that there are two longitudinal grooves above the foramen interventriculare, one above the fascia dentata, the other below it. Compare Elliot Smith's figure of the brain of *Ornithorhynchus* ('98, fig. 2). The lower of these sulci separates the fascia dentata from the fimbria and is therefore the fimbriodentate sulcus. The upper one is the fissura hippocampi. The area parolfactoria is prominent but relatively smaller than in the turtle. The primordium hippocampi stretches forward from the dorsal commissure and together with the fascia dentata extends into the medial wall of the olfactory peduncle. Compare figures 25 to 28 and figure 86.

These structures have been studied in a number of mammals. Figures 23, 42, 50, 60, 67, 73 show that in the rat, rabbit, striped gopher, bat, mole, and bear the relations are essentially the same

as in the opossum. The same is true in the sheep and foetal dog. The area parolfactoria is smaller in all of these than in the turtle or the opossum, and in some other mammals examined (cat, adult dog, man) this ridge fails to appear in the ventricle. This is due in part to its smaller size (man especially) and in part to the secondary obliteration of the ventricle between the area parolfactoria and the head of the caudate nucleus. In these forms the primordium hippocampi seems to extend down in the medial wall to the floor of the ventricle.

INTERNAL STRUCTURE OF THE MEDIAL WALL

To illustrate the relations in the internal structure of the medial wall of the hemisphere several transverse sections are drawn from the brains of the turtle, opossum, bat, rabbit, rat, mole and bear.

Section through the olfactory peduncle. Figures 12, 25, 41, 48, 56, 65, 75. The matter of interest in this section is the fact already well known from the work of Elliot Smith and others, that the hippocampal formation extends rostrad almost to the formatio olfactoria in most reptiles and lower mammals. The sections selected do not pass through the extreme rostral end of the hippocampal formation but are taken either through the olfactory formation or very close caudal to it. In each section there is seen beneath the ventricle the head of the caudate nucleus, covered ventrally by a thicker or thinner tuberculum olfactorium. The cells of the tuberculum extend up a short distance into the medial wall. Dorsal to this appears a mass of cells which are without regular arrangement and usually are smaller and paler than those in adjacent nuclei. This is the body which has been identified in the above pages as the primordium hippocampi. Above this is a dense mass of deeply staining cells which is continuous caudad with the supra-callosal hippocampus in mammals and with the medio-dorsal cortex in reptiles. This is the rostral end of the hippocampal formation.

Section near genu corporis callosi. Figures 42, 50, 58, 66, 75, 77. Since it is impossible to compare any particular section of the turtle's brain with a section through the genu of the corpus

callosum in mammals, the attention of the reader is called to figures 12 to 16, representing sections of the brain of the turtle rostral to the commissures. They are sufficiently described in the explanation of the figures.

In the mole, bat, rat, gopher, and rabbit the precallosal hippocampus approaches the ventral aspect of the genu and continues caudad beneath the corpus callosum to become lost in or fused with the underlying septum pellucidum (undifferentiated primordium hippocampi). This will appear more clearly in the description of sagittal sections below.

As is well known, the hippocampal formation also continues caudad over the corpus callosum. Figures 42, 50, 58 and 66 show the hippocampal formation as it bends around the genu. It is accompanied by precommissural fibers of the fornix system some of which come from farther rostrad while some come up through the septum pellucidum to course around the genu. Below the hippocampal formation and occupying the whole thickness of the wall is the primordium hippocampi. It is small in the rabbit, very large in the mole and in the bat. Below this are the nucleus parolfactorius medialis and nucleus parolfactorius lateralis. Examination of sections farther forward as well as those now under consideration, shows that the nucleus lateralis is in direct continuity around the ventral angle of the ventricle with the nucleus caudatus. The two bodies seem to have the same structure and to form parts of one mass. This is a very conspicuous fact in all the forms examined and has already been noted by Unger and by Kappers. The boundary between the lateral nucleus and the primordium hippocampi is marked by a slight ventricular sulcus, which has been described above from dissections, and a well marked cell-free zona limitans. This zona limitans does not cross the medial wall directly, but at this level appears as a semicircular line of division between the nucleus parolfactorius lateralis and nucleus caudatus internally and the superimposed tuberculum olfactorium externally. Just medial to the zona is seen in most lower mammals either an incomplete and irregularly broken plate of darkly staining cells (Nissl preparations) or a few isolated masses of such cells. These are

the islands of Calleja and it is readily seen that they belong with the tuberculum olfactorium in which such islands are numerous. I have never seen these islands rising in the medial wall quite as high as the nucleus parolfactorius lateralis. Medial to these is a more diffuse layer of cells which is continuous ventrally with the superficial layer of the tuberculum external to the islands. This is the nucleus parolfactorius medialis. It is evident that both this and the islands constitute a continuation of the tuberculum olfactorium into the medial wall, as the writer has pointed out in the case of selachians ('11 a). The boundary line between the primordium hippocampi and these two nuclei in the medial wall is somewhat V-shaped as indicated in the figures. The medial nucleus varies greatly in extent but its cells are always imbedded among the fibers of the fasciculus prae-commissuralis, forming a more or less dense superficial plate or layer. In the mole and also in the rabbit it comes up from in front almost to the fornix columns; in the bat it rises somewhat higher than the nucleus lateralis, but a large primordium hippocampi intervenes between it and the fornix and the corpus callosum. In the rat the nucleus medialis is small and does not extend as far rostrad as in most forms. In the turtle the nucleus medialis covers the outer surface of the nucleus lateralis with diffusely scattered cells as in the selachian and frog.

The section through the genu in the bear's brain presents a very different appearance from those described above, owing to the great development of the frontal lobe which occupies the space in this section between the olfactory bulb and the genu. In figure 73, however, is drawn a section between the genu and lamina terminalis in which the relations of the primordium hippocampi, nucleus parolfactorius lateralis and nucleus parolfactorius medialis are seen to be as described above. In this section the indusium appears above the corpus callosum and the corresponding cord of gray beneath it.

Section through the neuroporic recess. Figures 17, 29, 44, 51, 62, 69, 70, 72. This section cuts at the same time the anterior commissure, the fornix columns and some part of the anterior pallial commissure complex, and passes just rostral to the fora-

men interventriculare. Some attention must be given separately to each of the forms studied.

In the turtle (figs. 9 and 17) the neuroporic recess is nearly obliterated in the adult, apparently through the anterior and pallial commissures approaching one another in later embryonic stages. The dorsal commissure has the form of a loop whose two limbs rise dorsally at either side to reach the hippocampus (figs. 9 and 16). All recent authors agree that the compact layers of cells occupying the medio-dorsal wall of the hemisphere represents some part of the hippocampal formation of mammals. Meyer ('92), Elliot Smith ('96) and Levi ('04) rightly hold that the lower portion of this cortex is the forerunner of the fascia dentata. The ventral boundary of the hippocampal formation is sharply marked both by the sudden change to a body containing cells irregularly scattered through the thickness of the wall and by a well-defined sulcus. The mass of scattered cells is the primordium hippocampi above described. Its dorsal portion in this section is filled by fibers of the fimbria system. This is an old system of fibers connecting olfactory centers in front with the whole length of the hippocampus. It is well developed in the embryo before the hippocampal commissure is formed. The constitution of this precommissural representative of the fimbria is discussed below. The sulcus above mentioned, since it lies between the fimbria and the developing fascia dentata, must be regarded as the homologue of the fimbrio-dentate sulcus of mammals. This sulcus has been variously treated by previous authors. Elliot Smith ('03) calls it the sulcus limitans in *Sphenodon*, Edinger ('96) and Unger ('06) call it the fissura arcuata, Kappers and Theunissen ('08) call it fissura septo-corticalis, de Lange ('11) uses the same name but calls it also fissura arcuata. Herrick ('10) discusses the matter, and retains the term fissura arcuata. The sulcus in question can not be the fissura arcuata, since that is situated within the hippocampal formation, dorsal to the fascia dentata. The fissura arcuata is not present in the reptiles studied by the writer. On the other hand, the fimbrio-dentate sulcus is a constant feature in the brains of reptiles and mammals. It is the sulcus shown by Elliot Smith ('98, pl. XI,

'10, fig. 5) in the brain of *Ornithorhynchus* beneath the fascia dentata and seen in the same position in the brain of *Didelphys* and the bat (figs. 22, 30, 43, 44). Elliot Smith ('03, p. 469) gives this sulcus the name *sulcus limitans hippocampi* and points out the error of writers on the reptilian brain who regard this as the *fissura arcuata*. In higher mammals the growth of the corpus callosum greatly disturbs this sulcus in the rostral half of the hemisphere but the *fimbrio-dentate sulcus* is a well known landmark in that region of all mammalian brains where the primary relations of hippocampal formation and fimbria are retained. It persists throughout the length of the corpus callosum in the bear (fig. 76) and the bat (figs. 43, 44).

The sections of the turtle brain show that the body which Meyer called *septum pellucidum* and which I have here called *primordium hippocampi* is marked off from the area *parolfactoria* by a ventricular sulcus and a cell-free zone, is traversed by the olfacto-cortical fibers and by the hippocampal commissure. This body lies above the neuroporic recess and in all these respects it agrees with the *primordium hippocampi* of selachians. The only difference is that a part of the hippocampal *primordium* of selachians has developed into hippocampal cortex in reptiles.

In *Didelphys* (fig. 29), as in the marsupials described by Elliot Smith, the dorsal or anterior pallial commissure lies in the same plane with the large anterior commissure and above the neuroporic recess. The mass of gray matter in which it is partly imbedded projects into the ventricle and is separated from the *parolfactory nuclei* rostrad by the ventricular sulcus *limitans* in the manner described above. It is the residue of the *primordium hippocampi* of selachians and is continuous with the fascia dentata and hippocampus above. The fibers of the anterior pallial commissure bend dorsad through the *primordium* to emerge on the ventricular surface of the hippocampus as the *alveus*. Above the commissure is the *recessus superior* whose membranous walls are attached to the dorsal surface of the commissure. This is nothing more or less than a rostral pocket of the third ventricle covered by *tela chorioidea*, formed by the commissure pushing

into the ventricle as a transverse ridge. Just lateral to the attachment of the tela is the fimbrio-dentate sulcus. Above the fascia dentata the deep fissura hippocampi presents the typical mammalian relations. The constitution of the anterior pallial commissure is discussed in a later section.

In the bat (fig. 44) the section cuts the two commissures very much as in the opossum. Above the anterior pallial commissure is a well developed hippocampus and fascia dentata. The hippocampal fissure and the fimbrio-dentate sulcus are similar to those of *Didelphys*. The lateral ventricles are reduced to very narrow slits. The medial wall of the ventricle consists of a primordium hippocampi in which the pallial commissure is imbedded as in all other forms.

In the mole (fig. 51) the section cuts the large anterior commissure, the fornix columns and the corpus callosum. Above the corpus callosum is the indusium with the stria Lancisii, and beneath it the primordium hippocampi which is relatively larger than in any other mammalian brain studied. Between the fornix columns it contains large cells comparable with the large pyramids of the hippocampus. The relations of the neuroporic recess are commented upon in connection with the sagittal section, which shows them better.

In the rabbit (figs. 69, 70) the general relations are the same as in the mole. A very small indusium appears above the corpus callosum. The septum pellucidum of authors consists of a diffuse gray mass which is directly continuous forward with the primordium hippocampi. Just rostral to the neuroporic recess each lateral half of this primordium is almost semicircular in outline (fig. 69). The fornix columns come up through it, a large part of their fibers turning laterad as the body of the fornix to become continuous with the fimbria, another large part ascending to take a place immediately beneath the corpus callosum near the median line in which position the fibers continue caudad to be distributed to the hippocampus. This is the fornix superior of Kölliker and Elliot Smith. The fornix body divides at the level of the neuroporic recess into dorsal and ventral portions. The dorsal portion forms a thin covering for that part

of the hippocampus which extends farthest rostrad beneath the corpus callosum (hippocampal flexure). This appears in sections immediately behind the foramen and thus overlaps dorsally the primordium hippocampi in which the commissure is imbedded. The ventral portion becomes the fimbria proper.

In the bear (fig. 72) the neuroporic recess projects rostrad between the fornix columns somewhat beyond the rostral border of the anterior commissure. Above the recess the space between the fornix columns is occupied by the hippocampal commissure. Above this are the bundles of the fornix superior and among them a little gray matter which is the continuation of the subcallosal hippocampus traced back from the level of the genu. At about this level this small mass of gray becomes fused with the primordium hippocampi (septum). The latter body is here largely filled with the fibers of the fornix system. Its ventricular portion is relatively free from medullated fibers. On the dorsal surface of the corpus callosum is the indusium with the striae Lancisii which will be more fully described in a later section.

The rat possesses the largest hippocampal formation rostral to the foramen interventriculare that the writer has seen in any mammal. At the olfactory peduncle (fig. 56) a very broad area of deeply staining cortex is seen on the medial surface almost in contact with the olfactory formation. This is reduced to a narrow band at the level of the rostral border of the caudate nucleus (fig. 57). Here is a great development of the deep layer of the tuberculum and between this and the cortex mentioned is a narrow area of small pale cells which represent the primordium hippocampi. At the rostral border of the corpus callosum (fig. 58) the special enlargement of the deep layer of the tuberculum has disappeared and the islands of Calleja invade the lower part of the medial wall. The primordium hippocampi is much increased in size while the band of cortex previously described is still small. Curving about the genu of the corpus callosum is seen well developed cortex accompanied by the stria medialis Lancisii. Nine sections farther caudad (fig. 59) the two bands of hippocampal cortex have united into a broad band

beneath the corpus callosum, and above the latter is a rather large indusium. The primordium hippocampi is broad and is bounded below by very large islands of small cells which take a very deep stain. At this level the primordium hippocampi includes a large mass of gray between the sub-callosal hippocampal cortex and the ventricle. It is separated from the nucleus parolfactorius lateralis by an oblique zona limitans. The nucleus lateralis appears to be merely the medial part of the head of the caudate nucleus which does not extend into the medial wall beyond the lower angle of the ventricle. This relation is more striking in the rat than in any other mammal studied. Fifteen sections caudal to the last figure the very broad sub-callosal hippocampal cortex has become reduced to a small band (fig. 60) but the primordium is still large and bears the same relation to the nuclei parolfactorii medialis and lateralis and to the islands of Calleja as in other forms described above. Caudal to this (fig. 61) the cortical band merges into the primordium, which maintains the usual relations. At the level of the neuroporic recess (fig. 62) all the relations are closely similar to those in the rabbit. Caudal to the foramen the primordium extends a short distance among the fimbria fibers and then is continued as a band of cells flattened between the hippocampal commissure and corpus callosum, accompanying the fornix superior as in the rabbit and bear. These cells eventually are lost to view among the cells of the deep or ventricular layer of the hippocampus.

Relation of primordium hippocampi to true hippocampus caudal to the foramen interventriculare. The fact has been mentioned above that in the rabbit the hippocampus extends rostrad beneath the corpus callosum almost to the level of the foramen and overlaps the primordium hippocampi. The commissure and fornix are imbedded in a continuous mass of gray which has reached the cortical stage of differentiation behind the commissure, while in front it remains an undifferentiated primordium. The same is true of the mole. Here the hippocampus proper extends rostrad only a short distance beneath the splenium (fig. 88) but the primordium continues caudad over the foramen

among the fimbria fibers and as a mass of pure gray matter on the ventricular surface of the fimbria until it overlaps the rostral end of the hippocampus. Here the primordium and the cortex meet as a common bed for the fimbria and the caudal portion of the hippocampal commissure which lies beneath the splenium. The condition in the rat also indicates the essential continuity of the primordium hippocampi and the hippocampus proper. This subject is more fully discussed in the following section.

In the turtle (fig. 18) the primordium hippocampi behind the foramen is small and is occupied by the fimbria. The fimbriodentate sulcus continues back in the medial wall of the hemisphere as far as the fimbria can be recognized, some distance beyond the end of the choroid fissure (fig. 20).

EXAMINATION OF MEDIAL SURFACE OF HEMISPHERES AND OF SAGITTAL SECTIONS

The medial surface of the opossum's hemisphere is drawn in figure 22. The reader will notice the deep hippocampal fissure above the fascia dentata and that it is suddenly obliterated rostrad by the deep in-folding of the dorsal wall at the sulcus olfactorius. The fascia dentata is narrow over the anterior pallial commissure and grows wider rostrad. Beneath it is the broad bundle of precommissural fibers of the fornix system. The anterior pallial commissure stands in the path of these fibers. The fimbriodentate sulcus begins between the dorsal end of these fibers and the fascia dentata and extends caudad over the commissure. The locus of the neuroporic recess is clearly seen in the so-called recessus inferior. Upon this surface no sulcus is visible between the primordium hippocampi and the area parolfactoria. It has probably been obliterated by the great bundles of precommissural fibers running dorso-ventrally across it. The course of the boundary line, as indicated by the internal structure above described, is shown in figure 86.

In figures 35 and 34 are drawn a nearly median sagittal section through the region surrounding the commissures and a section of the anterior pallial commissure and hippocampal formation lateral to the median plane. In the most medial section it

is seen that the pallial commissure lies in a lamina supraneuroporica containing gray matter and that the tela is attached to the dorso-rostral border of this, forming the recessus superior. The projection of the chief volume of the lamina supraneuroporica into the ventricle is a characteristic difference between marsupials and higher mammals. In the bat (fig. 45) an intermediate condition is found. The pallial commissure has already the crescent shape which is characteristic of those forms in which the corpus callosum has begun to separate from the hippocampal commissure. In the caudal thick part of the commissure are found a number of small bundles of non-medullated or lightly medullated fibers and the rostral part of the crescent seems to contain many lightly medullated fibers. This rostral horn of the crescent of course forms the precommissural portion of the alveus. Whether the lightly medullated fibers have different functions from the heavily medullated can not be decided at present. In the section lateral to the median plane it is seen that the primordium hippocampi is directly continuous with the fascia dentata through the commissure bundles (figs. 33, 34). The cells of the fascia dentata with which the primordium comes into relation are those in the concavity of the fascia dentata which Kölliker ('96, p. 737, fig. 777) and Edinger ('04, p. 333 and fig. 232) call the end-portion of the layer of pyramidal cells of the hippocampus. In transverse sections this continuity of the primordium with the hippocampal formation is not conspicuous because the alveus seems everywhere to form a complete partition of fibers between the two gray masses. In sagittal sections it is seen at once that the alveus is in bundles between which columns of cells connect the two masses; or, rather, that the two form one continuous mass which is traversed by the alveus bundles.

When transverse sections stained by a cell stain are studied more carefully instructive facts are brought out. First, as is well known from the work of Elliot Smith and Levi, the fascia dentata in its rostral part is directly continuous with the ventral border of the hippocampal cortex, as seen in a transverse section (fig. 28). In both hippocampus and fascia dentata is

seen a sparse layer of polymorphous cells next to the ventricle, in part mingled with alveus fibers. In the rostral part of the fascia dentata this ventricular layer consists of a much larger number of cells and these are broadly continuous with the mass of the primordium hippocampi (figs. 28, 32). Further caudally, where the fascia dentata becomes displaced so as to embrace the border of the hippocampus in its concavity, the cells which fill this concavity are seen in favorable sections in direct continuity with the primordium through the alveus (figs. 29, 30, 31). From these facts it appears that the polymorphous layer contains the primitive cells of the hippocampus and is comparable to the primordium hippocampi. This is especially clear in the relations of the primordium to the hippocampal cortex in the pre-callosal region in the rat (figs. 58, 59).

The brain of the bat is rather small for dissection and the writer has not had a sufficient number of specimens to warrant using them in this way. Therefore the median plane of this brain is reconstructed from sagittal sections and a parasagittal section is drawn to illustrate the arrangement of centers in the medial wall (figs. 45, 46). These figures show clearly that while the nucleus parolfactorius medialis extends high up toward the pallial commissures it does not reach them but these commissures are imbedded in a considerable mass of gray matter which lies dorsal to the level of the neuroporic recess. This mass is the primordium hippocampi and is separated from the area parolfactoria below by a well marked cell-free zona limitans hippocampi. The primordium consists of two parts, a dense collection of small cells forming a bed for the hippocampal commissure (fig. 46), and a much less dense area of larger cells beneath the corpus callosum. Rostrad the zona limitans rises almost to meet the genu of the corpus callosum, but in sections to one side of the median plane (fig. 47) the supra-callosal hippocampus (indusium) surrounds the rostral border of the commissure and becomes continuous with this sub-callosal portion of the primordium hippocampi. Rostral to the genu the hippocampal formation extends to the olfactory peduncle. Close to and in the median plane the large celled portion of the primordium is reduced to small size but is distinct from the nucleus parolfac-

torius medialis. Caudally the commissure is imbedded between the primordium and the hippocampus proper and dorsally the cells of the indusium are occasionally found intermingled with the bundles of the corpus callosum (fig. 47) so as to establish a continuity between the indusium and the primordium. In front of the commissure there is a broad continuity of these bodies as already described. It thus appears that the corpus callosum and hippocampal commissure are imbedded in a continuous mass of gray matter which occupies the thickened lamina supraneuroporica and extends into either hemisphere as the hippocampal formation. At the point *m* where the tela chorioidea is attached to the caudal surface of the hippocampal commissure is a prominent module of cells to which Elliot Smith ('97 e) has called attention in *Nyctophilus*. This is the primary upper border of the lamina supraneuroporica and the cells belong to the indusium verum as defined by Professor Smith ('97 e). It might be called *nodulus marginalis*.

In the rat there is a large corpus callosum with well developed genu and splenium. The hippocampal commissure is a plate of fibers broad dorso-ventrally and standing nearly vertically beneath the body of the corpus callosum, a little nearer to the genu. At its dorsal border the plate of fibers bends caudad beneath the corpus callosum and becomes continuous with the thin edge of the splenium (fig. 89). This form of the commissures is apparently due to the dorsal end of the large hippocampus which fills the angle between the hippocampal commissure and the splenium, the hippocampal flexure of Elliot Smith. The pillars of the fornix are large and rise in front of the hippocampal commissure relatively free from mingling with it (fig. 64). Just beneath the corpus callosum they turn latero-caudad in the fimbria. Between the fornix columns and the hippocampal commissure, and to some extent mingled with the latter, is the small-celled nucleus already described in the bat. Beneath the rostral part of the corpus callosum the septum pellucidum is filled with large cells as in the bat. Here in the rat these cells become more compactly arranged near the median line and are directly continuous with the indusium around the genu as described above from transverse sections. Rostral from the genu

the indusium is continued by a cortical band which reaches to the olfactory bulb. Beneath this is a band of lightly staining cells, loosely and irregularly arranged, which represents the primordium hippocampi. It is directly continuous with the less compact part of the septum pellucidum above described. Finally, this part of the septum pellucidum in its caudal and lateral part is connected with the hippocampus proper by columns of cells between the bundles of the hippocampal commissure as in the opossum. See the description of figures 63 and 64. There is therefore a continuous formation which begins at the olfactory peduncle, divides at the genu into indusium and septum pellucidum and unites again in the hippocampus behind. In other words, the hippocampal and callosal commissures are imbedded in one formation which in part is developed into hippocampus and in part remains in the primitive condition. The septum pellucidum includes the larger part of this undeveloped primordium hippocampi.

The median plane of the brain of the mole is reconstructed in figure 55. There is an enormous massa intermedia and the third ventricle between it and the anterior commissure is reduced to a very narrow slit. The hippocampal commissure is nearly horizontal in position and the septum pellucidum very narrow dorso-ventrally. It is largely occupied by fibers of the fornix superior. The feature of really striking importance about this section is the existence of a band of non-nervous tissue leading from the neuroporic recess out to the surface below the genu of the corpus callosum. In Weigert sections this has almost the appearance of a canal and is occupied by blood vessels. In the sections at either side of the median plane the fibers of the precommissural bundle, which are interrupted in this figure, pass on into the fimbria. When this is compared with the condition in the mammalian embryo it is evident that this is a vestige of the great sagittal fissure which has been closed up by the encroachment of the paraterminal body below and the pallial commissures above. If this figure be compared with the sagittal sections of the bat's brain, it will be seen that a quite similar line of demarcation between the paraterminal body and the septum occurs in the bat and that the cell-free space is occupied by blood vessels.

Werkman's photographs of sagittal sections of this region in bat embryos are extremely instructive in this connection (Werkman '13, figs. 13, 17 and 18). These show the relations between the paraterminal body and the septum in three stages. Essentially the same condition is seen in the embryos of the rabbit, cat and other mammals. If now we turn to the selachian and compare Werkman's figure 18 and figures 45 and 55 of the present paper with the median section of *Scyllium* (fig. 84) we find an almost complete correspondence of part for part. The external neuroporic recess in the selachians is a deep canal containing blood vessels. In some selachians the canal is obliterated and the vessels are imbedded in a solid mass of tissue. An external pit is retained in the adult bat and mole and in all embryos. In the bat and mole a cell-free and fiber-free band occupied by blood vessels marks the position of the external neuroporic recess. The same is true but less prominent in some other mammals. In the human embryo of 31 mm. the external neuroporic recess is penetrated by a special group of blood vessels (fig. 8). The mole and the bat show in a striking manner that even when there is a large corpus callosum, as in the mole, the essential relations of the area parolfactoria and the hippocampal formation with its pallial commissures do not differ at all in selachians and mammals.

One other point in the brain of the mole may be mentioned here. When the region of the splenium is studied in transverse sections it is seen that the enlargement of the indusium behind and beneath the splenium (*fasciola cinerea*) consists of much larger cells than those in the adjacent hippocampus proper (fig. 54). These cells form a plate which is continuous at its lateral edge with the layer of pyramids of the hippocampus. The *fascia dentata* takes no part whatever in the formation of the indusium. As the hippocampus is followed forward beneath the corpus callosum the cells of the *fasciola cinerea* gradually blend to some extent with those of the hippocampus. This plate of cells is, however, readily followed forward beneath the hippocampal commissure until the *fascia dentata* disappears from the section and this plate of cells merges with the septum pellucidum as above described (fig. 53).

CONSTITUTION OF DORSAL COMMISSURE

Symington ('93) and Elliot Smith ('97 d, '02, '03 b) have presented evidence that the marsupials do not possess a true corpus callosum. The corpus callosum of mammals is defined as consisting of fibers coming from undoubted neopallial areas, invading the alveus of the hippocampus and crossing to the opposite hemisphere through the dorsal commissure. Elliot Smith ('03) believes that while such a commissure does not exist in marsupials, the function of a corpus callosum is performed by fibers which cross in the anterior commissure, reaching it by way of the external capsule. The writer has at hand at the time of writing only Smith's reply to Zuckerhandl (Smith '03 b). As this clearly defines the questions involved it will be of interest to note some evidence bearing on the subject derived from the brain of the opossum. In Weigert sections corresponding to the one from the brain of *Perameles* beautifully figured by Elliot Smith the writer can not doubt that fibers enter the alveus from the medio-dorsal cortex far beyond the transitional area between hippocampus and general cortex (fig. 37). Sagittal sections show this more clearly (fig. 39). Since Weigert sections are not conclusive on a point like this, an attempt was made to test the presence of callosal fibers in the dorsal commissure by experiment. The attempt in two operations to cut the dorsal commissure without injury to anything else failed, but in other experiments light was thrown on this question. In one animal an area of dorsal cortex (shown in fig. 40, *a*) was scraped out. The animal when killed showed no infection and there is in the sections no evidence of any injury being done to the hippocampus or alveus. Preparations were made by the Marchi method and showed the following results: internal capsule deeply degenerated; external capsule affected; anterior commissure not affected at all; alveus and dorsal commissure contain many degenerated fibers, traced from the lesion (fig. 40).

In another animal a much larger area of dorsal cortex was destroyed (fig. 40, *a*). Although the internal capsule was badly degenerated and the external capsule likewise, no degeneration

is seen in the anterior commissure. The dorsal commissure shows degenerated fibers, but in this case there was possibility of direct injury to the alveus.

In a third animal in which a large lesion was made in the thalamus and midbrain, there was incidental injury to the dorso-medial angles of the hemispheres. On the left side this invaded the hippocampus secondarily, largely destroying its dorsal portion; on the right it affected only the dorsal cortex. The hippocampal commissure was of course deeply affected and both sides show degeneration in the internal capsule. The anterior commissure shows no degeneration. The character of the degeneration in the dorsal commissure differs as the lesion affects the hippocampus or the general cortex. After hippocampal lesion the degeneration is more profuse and the blackened droplets are much coarser. This is perhaps to be explained on the supposition that fibers arising in the general cortex are more lightly medullated. Attention has been called to the fact that the dorsal commissure contains bundles of lightly medullated and non-medullated fibers. A similar explanation can scarcely be given for the apparent absence of fibers from the dorsal cortex in the anterior commissure. One receives the impression from Weigert sections that the anterior commissure consists almost wholly of medullated fibers.

It thus appears that in the opossum we have positive evidence in Weigert and Marchi preparations for the presence of corpus callosum fibers in the dorsal commissure and negative evidence from Marchi preparations regarding fibers from the dorsal cortex crossing in the anterior commissure. Further study of the distribution of the anterior commissure is under way.

The facts above stated warrant a reinvestigation of the Australian marsupials. The presence of true corpus callosum fibers in marsupials and also in reptiles is to be expected in view of the fact that in fishes, amphibians and reptiles sensory radiations ascend from the thalamus to the telencephalon and of the further fact that in selachians the telencephalic center for these thalamic radiations is connected with its fellow by a commissure which bears the morphological relations characteristic of the

mammalian corpus callosum. It is worthy of note that Unger ('06) describes in the gecko a large part of the dorsal commissure going to the cortex far lateral to the hippocampus. These fibers can scarcely be other than corpus callosum fibers. Pedro Ramon ('94) has also figured fibers in *Lacerta*, which must be callosal fibers if the figure is accurate. See Elliot Smith's discussion of this ('03, p. 482). See also Cajal ('04, p. 1103), who states that these are callosal fibers. In the turtles studied the lack of medullation in the dorsal commissure has made it impossible thus far to secure positive evidence as to the presence of callosal fibers.

DEVELOPMENT OF PALLIAL COMMISSURES

The development of the pallial commissures has been the subject of extensive phylogenetic and ontogenetic studies by numerous authors. For the general course of evolution of the two commissures we are indebted chiefly to the comparative researches of Elliot Smith. The studies of embryonic development by Schmidt ('62), Mihalkovics ('77), Blumenau ('91), Marchand ('91), Martin ('93), His ('89, '04), Hochstetter ('98), Zuckerkandl ('01, '09), Grönberg ('01), and Goldstein ('03), have given conflicting results on certain points. Elliot Smith maintained that the fibers of the corpus callosum in mammals entered the commissure bed which already contained the hippocampal commissure, that the callosal fibers became segregated in the rostral limb of a crescent-shaped commissure, that the growth of the general cortex was followed by an increase of the callosal fibers, that these fibers caused an expansion and stretching of the commissure bed and that the entire hippocampal-callosal commissure system remains in higher mammals surrounded by the vestiges of the primary commissure bed. Mihalkovics, His and Zuckerkandl have held that the corpus callosum forms in a secondary area of fusion of the medial walls of the hemispheres. This is opposed by Goldstein who believes that in man the primary commissure bed is expanded by the callosal fibers growing into it. There has just come to hand as I write the study of Werkman ('13) who finds this to be true in *Vesperugo*, *Erinaceus*, and *Talpa*. The writer has studied carefully the development of the

corpus callosum in pig embryos and must agree with Goldstein and Werkman. The intermediate view of Marchand, that there is a very early fusion of a small area of the hemisphere walls which is thereafter stretched by the growth of the commissures may be explained by two factors. First is the thickening of the lamina terminalis (and of the lamina supraneuroporica as defined in the present paper) by the proliferation of neuroglia as described in detail by Werkman. This results in a thickened "Glia-schicht" through which the fibers may run. Second, there is a migration of cells (neuroblasts) into the lamina supraneuroporica from the hippocampal primordia at either side just as there is a secondary thickening of the lamina terminalis by migration of cells from the paraterminal body to form a bed for the anterior commissure. In figures 7 and 24 is seen a ridge extending upward and forward from the lamina supraneuroporica in the hemisphere wall. This ridge indicates the line along which pallial (hippocampal) commissure fibers enter the lamina and cells migrate into this lamina to form the commissure bed described above in several mammals. This thickening of the roof plate at first by glia and afterward by neuroblasts produces the massive lamina supraneuroporica which is characteristic of vertebrates and may explain Marchand's conception of an early area of fusion and Grönberg's *conrescentia primitiva*. Space can not be taken for a full account of the development of the commissure. The reader is referred to the description of figures 78 to 81.

That there is no fusion of the medial walls and that the commissure bed is merely expanded by intussusception of callosal fibers is evidenced by the appearance of the commissures in the lamina supraneuroporica (upper border of lamina terminalis of authors) (Marchand, figs. 7, 9; Werkman, figs. 5, 17, 24); the fact that the corpus callosum in all stages of growth has a smooth and regular border upon which the falx and anterior cerebral artery lie as if they had been pushed before the developing commissure (Goldstein, p. 46 and fig. 15); and the fact that the commissures are always completely surrounded by a thicker or thinner layer of nerve cells (Blumenau); which I can confirm

from my own studies. The last point is of great importance. It has been shown in a previous section that the corpus callosum and hippocampal commissure in the opossum, bat, mole, rat and rabbit are everywhere surrounded by the hippocampal formation and the primordium hippocampi. The cells of the indusium verum of Elliot Smith and the cells among which the commissure fibers are imbedded must be regarded as the remnant of the cells which enter the lamina supraneuroporica from the adjacent hippocampal primordia in early stages of the embryo. Thus the anterior pallial commissures in mammals are imbedded in the primordium hippocampi just as they are in selachians.

STRIAE LANCISII AND INDUSIUM

To the very concise and illuminating survey of the history of these structures given by Elliot Smith ('97 e) I can add a few interesting comments on the basis of the forms studied. Great differences appear in these structures in various mammals. In most of the rodents the indusium is small and the morphological arrangements seen in the better developed hippocampus are lost. In the woodchuck (*Arctomys*) there is a fairly thick band of cells continuous across the median line and covered by a layer of fibers. This probably indicates a fusion of the indusium and striae of the two sides. The striped gopher (*Spermophilus tri-decemlineatus*) agrees with the woodchuck. A very different condition appears in the opossum and the bear. I have seen no mention made of a stria Lancisii in the marsupials and none was to be expected on the hypothesis that this bundle represents fibers which run within the hippocampal formation when that is well developed. Elliot Smith states that in Eutheria "the vestigeal fascia dentata lies in the region of the stria medialis." I was surprised, therefore, to find in transverse sections of the opossum's brain well defined striae mediales on the dorsal surface of the pallial commissure. As shown in figures 29, and 30, these are good-sized dense bundles lying at either side of the middle line and separated from the fascia dentata by the fimbrio-dentate sulcus already described. Traced caudad these bundles curve down on the caudal surface of the commissure and turn laterad.

They are then lost in the commissure itself and presumably go to the hippocampus. Rostrally these fibers bend down over the rostral border of the commissure and are made up from the fibers of the fasciculus praecommissuralis (figs. 28, 35, 36). From the stria medialis in its longitudinal course small bundles are given off to the deep layer of the dentate fascia and the hippocampus (fig. 36). These are in all probability fibers from the tuberculum and other olfactory centers to the hippocampus. The fact that the stria is separated from the fascia dentata by the fimbrio-dentate sulcus at once suggests comparison with the fimbria in reptiles. The stria medialis undoubtedly corresponds to a part of the reptilian fimbria and it is important to notice that it runs in the primordium hippocampi (septum) in the opossum as in the reptile. The stria lateralis is represented in the opossum by the longitudinal fibers in the stratum zonale of the hippocampus as Elliot Smith has stated. In addition to longitudinal association fibers of the hippocampus, however, these bundles include many fibers which come up from the tuberculum through the rostral part of the primordium hippocampi. These are fibers of the same category as those which make up the stria medialis; both are olfacto-cortical fibers. These run near the lateral border of the hippocampus, while the stria medialis runs along its medial border. Both the olfacto-cortical and the association fibers are seen in the mole (fig. 48) but most of them end in the precallosal hippocampus and primordium because the supracallosal hippocampus is reduced to an indusium in the mole. In the rabbit the stria medialis curves around the genu and is lost among the fibers of the precommissural fasciculus rather far back beneath the corpus callosum.

In the bear the indusium is rather large and maintains the relations seen in the opossum. As seen in figures 76 and 77, the stria medialis is separated from the hippocampus by a fimbrio-dentate sulcus, the fascia dentata and hippocampus can be distinguished and in the rudimentary hippocampus are seen both the vestigial alveus and the superficial stria lateralis. These structures curve about the genu and are well preserved beneath the callosum until the hippocampus expands into a broader band reaching to the olfactory peduncle (figs. 77, 75).

In the bat also the stria medialis is made up of precommissural fibers and runs independently of the fascia dentata, separated from it by the fimbrio-dentate sulcus (fig. 43). Caudally the fibers pass into the lateral part of the nodule of cells at *m*, and are lost. Probably they turn laterad here in the hippocampal commissure.

The above facts seem to show conclusively that the indusium and stria lateralis Lancisii are strictly vestiges of the hippocampus and fascia dentata as those exist in the marsupials. The stria medialis, however, is a separate structure present in the marsupial and comparable with the fimbria bundle in the reptiles. Professor Smith ('97, p. 85) speaks of the stria medialis as "the fimbria of the dorsal hippocampus." It together with the pallial commissure belongs in the septum or undeveloped hippocampal primordium. The stria medialis is strictly a part of the fornix longus as defined by Elliot Smith ('97, p. 88) but differs from all the other fibers of the fornix system in that it does not pierce any part of the pallial commissure complex in order to reach the hippocampus. This is recognized by Elliot Smith in the passage referred to, which reads:

From this account of the arrangement of fibers in the ox-brain, it is evident that *all the longitudinal uncrossed fibers of the fornix break through some part of the great dorsal commissure* (psalterium, splenium, or corpus callosum, as that term is generally understood) *in order to reach the septum*. These fibers constitute the true *fornix longus*. The *fornix superior* consists of those fibers of the fornix longus which do not pass through the main mass of the psalterium, but break through a commissure of non-hippocampal or a mixture of the latter and hippocampal fibres (i.e., corpus callosum and its splenium).

A few fibers of the fornix do not pass through any commissure. In the marsupial (fig. 4a) these fibers spring directly from the most anterior part of the hippocampus, and pass downwards to their destinations in front of the commissures. The corresponding fibers in the higher mammal (fig. 2a) become pushed forward by the extending genu corporis callosi, but their essential disposition is unaltered, i.e., they spring from the anterior extremity of the stria medialis Lancisii.

This statement requires modification in that the stria medialis is connected with the caudal as well as the rostral part of the hippocampus. The opossum and the bat show this conclusively. It is probably for the very reason that the stria medialis is related

to the caudal part of the hippocampus that it is preserved at all in higher mammals. Certainly it curves about the splenium to enter the fully developed hippocampus in all the forms that I have studied. It is undoubtedly made up chiefly of the very primitive system of olfacto-cortical fibers which are already seen in (cyclostomes and) selachians and which constitute most of the fimbria bundle in the rostral part of the reptilian hemisphere. The fibers of this system which enter the rostral part of the hippocampus nearly all disappear in mammals possessed of a large corpus callosum so that the stria lateralis Lancisii consists chiefly of hippocampal association fibers and is sometimes difficult to recognize in these higher forms.

Elliot Smith ('97, p. 55) suggested that the fasciculus marginalis described by him in *Ornithorhynchus* is represented in higher mammals by the stria medialis. The fasciculus marginalis in the opossum goes in part into the stria medialis and in part into the "association bundle" in the stratum zonale of the hippocampus. Since the latter is practically lost in higher mammals it would be true to say that the fasciculus marginalis is represented by the stria medialis. On the other hand, the stria medialis is not wholly made up of the fasciculus marginalis, since it receives fibers from the other parts of the precommissural system.

Two other points are worthy of notice in this connection. First, the relations in the bear show clearly that the sulcus corporis callosi in higher mammals is the fissura hippocampi, strictly comparable to that of the marsupial or the bat. And this suggests that when the perforating fibers of the fornix superior appear to go to the cingulum they are in reality only taking a position along the lateral border of the reduced hippocampus. The hippocampus is sometimes enlarged and the hippocampal fissure better developed in front of the corpus callosum or beneath the genu, as in the bear and the rat (figs. 58, 77). Second, the recognition that the stria medialis is independent of the hippocampus proper affords us a definite boundary line in most mammals between the hippocampal formation and the residue of the hippocampal primordium (i.e., septum, pallial commissures and

fimbria system). This boundary line is marked by the fimbriodentate sulcus. This adds needed emphasis to the fact that the corpus callosum does not itself mark this boundary.

GENERAL RELATIONS OF FIBER TRACTS AND COMMISSURES IN THE SEPTUM OF VERTEBRATES

These relations are readily understood in view of the considerations in the last section and of the conditions in selachians. In these fishes (fig. 91) the pallial commissures are imbedded in the hippocampal primordium, the corpus callosum dorsal to the hippocampal commissure. Interwoven with both commissures are longitudinal fibers of the tractus olfacto-corticalis, while the columns of the fornix emerge from among the fibers of the hippocampal commissure to descend at either side of the neuroporic recess as in reptiles and mammals. When in these higher forms a part of the hippocampal primordium becomes hippocampus the commissures remain imbedded in the undifferentiated primordium and the longitudinal fibers reach the hippocampus through the residual primordium, retaining essentially the same relations among themselves. As the hemisphere elongates, the olfacto-cortical system takes on the form of a more compact bundle, the precommissural fimbria. This is joined by the fibers from the nuclei of the lateral olfactory tract running by way of the anterior perforated space and helping to form the fasciculus precommissuralis. This bundle contains according to Elliot Smith ('97) and Cajal ('04) many fibers which arise and others which end in the septum. The efferent fibers go in part at least to the hypothalamus. The fibers which end in the septum belong chiefly to the olfacto-cortical system. A study of the precommissural system in the opossum and the rabbit shows that the fibers come chiefly from the olfactory tubercle, anterior perforated space, nucleus of the lateral olfactory tract and perhaps the whole pyriform lobe. It is the great system of fibers by which impulses are carried from the area olfactoria to the hippocampal formation. Since many of these fibers end in the septum, the fiber relations of the septum are not unlike those of the hippocampus itself.

The relations of the commissures to the fimbria, fornix superior and the stria Lancisii are surprisingly primitive. In marsupials and lower mammals, as in selachians, the two commissures are interwoven with longitudinal fibers of the fimbria system (olfactocortical) on their way to the hippocampus. When the corpus callosum increased in size, separated from the hippocampal commissure and arched dorsally to form the splenium, it carried up on its dorsal surface such fibers as ran over it. These are the stria medialis Lancisii. The fibers which were interwoven with the callosal fibers have retained that relation and are the fornix superior and perforating fibers of Kölliker; the fibers (olfactocortical and fornix columns) which were primitively interwoven or intermingled with the hippocampal commissure have retained that relation and form the mammalian fimbria.

This interpretation is illustrated in figures 92 and 93 showing the relations of the fornix system to the commissures in the bat, which is intermediate between the marsupials and higher mammals, and in the rat, which presents a commissure system essentially like that of man. Figures 35 and 38, showing the disposition of fibers in the opossum should be compared with these.

REMARKS ON THIS REGION IN THE AMPHIBIANS

In the writer's study of the selachian brain ('11) comparisons were made with the brain of the frog with reference to the position of the primordium hippocampi and its boundaries. At that time the writer accepted the sulcus limitans of Elliot Smith ('03) as the limit of the hippocampal formation and believed it to be the homologue of the sulcus which marks the boundary of the pallium in selachians. On this basis figure 75 of that paper was constructed. As has been stated elsewhere in this paper, the selachian sulcus is not the equivalent of the sulcus limitans of Smith in the reptile, while that in the frog is the same.

Anyone who will read the summary of errors and confusion in the literature of the amphibian septal region given by Elliot Smith ('03, p. 495) will understand that it would be useless for me to review this literature, especially as the result would be to note several additions to the list, made by later authors.

In the medial wall of the frog brain the sulcus and cell-free zona which have commanded the attention of all workers seems at once to divide the pallial from the basal areas and to correspond to the sulcus and zona limitans hippocampi in the selachian brain. It must be noticed, however, that a zona limitans, whether accompanied by a sulcus or not is usually the expression of a rearrangement of neurones in the adjacent areas due to functional or mechanical causes or both. In the selachian the zona limitans in the medial wall marks the line of meeting of simple olfactory with the olfacto-gustatory correlating centers. The line of demarcation between the two was perhaps determined by the connection of the neural tube with the ectoderm at the neuroporic recess. In the amphibian a second differentiation has begun and is well advanced in the frog, namely, the formation of hippocampus. This is marked by a change of form and rearrangement of cells in the pallium. The cells are now pyramidal and have their long axes placed vertically to the brain surface instead of being wholly without arrangement. Such a change constitutes a very marked difference from the selachian brain and leads to the formation of a new zona limitans. Below this zona limitans the neurones are scattered without any regularity. These facts, however, are not sufficient to show that the zona limitans separates pallial and basal areas. Immediately below the zona limitans appears a group of large cells which stand out conspicuously from the cells of the remainder of this region ('11 a, fig. 75, Herrick '10, fig. 40, *n. medialis septi*). When these cells are examined it is found that they form a column extending forward to the olfactory peduncle. This column of cells corresponds to the "septum" or primordium hippocampi of the turtle's brain. In the rather broad and shallow sulcus between it and the hippocampus runs the fimbria, as is well known.

Traced caudally this column of cells continues above the foramen interventriculare where it is separated from the developing hippocampus by a ventricular sulcus and a cell-free zone. This portion is known as the pars fimbrialis (Kappers) or the supra-foraminal portion of the paraterminal body (Herrick). The fimbria runs in this body to be distributed to the posterior part

of the hippocampal formation and in it the hippocampal commissure collects before it descends in the lateral wall behind the foramen. With the exception of the behavior of the hippocampal commissure the relations in the frog agree with those in the turtle. Behind the foramen it is not difficult to see that this body corresponds to the small primordium hippocampi in this position in the turtle and the sulcus along (beneath) which the fimbria runs is the fimbrio-dentate sulcus. Rostral to the foramen in the frog there is wanting the sulcus which separates the primordium hippocampi from the area parolfactoria in reptiles and mammals. Gaupp ('97, figs. 26, 28, 29) describes and figures a narrow cell-free zone between this nucleus and the cells below. He calls this column "ganglion septi," but it is entirely different from the nucleus septi of Unger, Kappers and others. The entire lower half of the medial wall has been assigned by most authors to the paraterminal body and it has been assumed that the paraterminal body extends up over the foramen.

Now this assumption is one of the most inexplicable things in the literature of forebrain morphology. It is quite possible to understand how the great growth and arching dorsad of the corpus callosum in mammals results in stretching up the commissure bed as set forth by Elliot Smith. And so long as it was thought that the commissure bed belonged below the neuroporic recess and was related to the lamina terminalis there was no escape from the logic of the argument so far as applied to mammals, that the paraterminal body had been stretched up into the supraforaminal position as the septum. But in reptiles and amphibians there is no such voluminous corpus callosum. There is therefore absolutely no motive for the extension of the paraterminal body up over the foramen. In amphibians there is not even a hippocampal commissure extending up over the foramen from in front. Moreover it is known that in reptiles at least the bed of the pallial commissures is supraneuroporic in origin. The supposition that a part of the paraterminal body has migrated into the caudal part of the hemisphere above the foramen is not only not supported by evidence but is quite unthinkable in view of all the known facts.

The writer would substitute for this view the following simple explanation of the septal region in the frog. Above and caudal to the foramen the medial wall is all pallial. A part of this pallial area has developed pyramidal cells and may be recognized as a true hippocampus in a low stage of organization. The remainder has retained the irregular arrangement of cells seen in the selachian pallium and gives passage to the fimbria and fornix fibers as in the reptile. Rostral to the foramen there has been the same development of hippocampus but the residue of hippocampal primordium is smaller and the line of demarcation between this and the area parolfactoria has been obscured in part. At the same time a new sulcus has developed in the medial surface. This sulcus forms along the line of demarcation between the pyramidal and irregular cells. The elongation of the hemisphere has led to the formation of the fimbria just below this sulcus, through the collection of many fibers of the olfacto-cortical system, on their way to the caudal part of the hippocampus. This sulcus is therefore the fimbrio-dentate sulcus.

Each element in the above explanation is perfectly simple and direct. There is no supposition that great masses of cells have migrated half the length of the hemisphere without motive. The only point of obscurity is the line of separation between the primordium hippocampi and the area parolfactoria. Since this is perfectly clear in reptiles and mammals it is quite legitimate to accept the hypothesis that in the frog it has lost somewhat in definiteness because of the small volume of the residual primordium hippocampi.

GENERAL OBSERVATIONS AND SUMMARY

When the results of the present series of studies are reviewed it is seen that great confusion prevails in the morphological conceptions and the nomenclature of this region of the brain. In these studies for the first time there has been built up a connected account of the evolution of the telencephalon beginning with primitive brains and taking into consideration the factors and processes by which the form of the mammalian telencephalon have been determined. Briefly summarized, these processes are

the following: (a) The olfactory placode retards the closing of the neural tube, causing the formation of the anterior neuropore. The neuroporic recess, seen in most vertebrate brains, marks the dorsal or caudal border of this neuropore. (b) Early in vertebrate history the somatic sensory nerve of the telencephalic segment is greatly reduced or disappears while the olfactory nerve is very large in lower vertebrates. No motor nerve is present in this segment. (c) The olfactory fibers enter the visceral sensory column in the telencephalon and cause a great hypertrophy of this column, which rises up in the brain wall and pushes the somatic sensory column to the lateral surface ('11 a, p. 41; '11 b, p. 497, 513-517). (d) The hypertrophy of the visceral sensory columns together with the slight growth of ventral columns near the optic chiasma has produced a forebrain flexure such that the visceral sensory column takes the form of a letter U. The basal limb of the U is occupied by secondary olfactory centers, its dorsal limb adjacent to the diencephalon by the olfacto-gustatory correlation center ('12 b, p. 369). The formatio olfactoria is situated in the base of the U and receives the olfactory nerve. The space between the limbs of the U is filled by somatic sensory area. (e) The evagination of the hemisphere begins first at the formatio olfactoria, involves gradually the olfactory bulb and later the olfacto-gustatory correlation center and finally the somatic sensory area ('11 a, p. 42; '12 b, p. 363). The evagination does not modify the fundamental relations. (f) The absence of a primary somatic sensory nerve in the telencephalic segment left the somatic sensory column free to serve correlating functions for cutaneous, kinaesthetic, visual and other somatic impulses. This has been the controlling factor in the development of the general cortex ('10 b). The assumption of terrestrial life has led to the rapid development of this somatic area, and its expansion has pushed the secondary olfactory centers and olfacto-gustatory center into the positions occupied in mammals by the pyriform lobe and the hippocampal formation respectively. (g) Interrelations between the somatic area and the several regions of the visceral column have resulted in the development of special centers; from the larger part of the olfacto-gustatory center a hippocampus; from

contiguous parts of the medial olfactory nucleus and somatic area, the corpus striatum; and from contiguous parts of the lateral olfactory nucleus and somatic area, the pyriform lobe. The details of all these processes remain yet to be worked out.

In the present paper we are concerned chiefly with the relations in the medial wall of the hemisphere and not with the general cortex or the pyriform lobe. The main questions at issue are the identification in reptiles and mammals of the basal olfactory center (medial olfactory nucleus or area parolfactoria) and the equivalent of the massive roof of the selachian telencephalon, the boundary line between the two, and the position of the forebrain commissures with relation to these two bodies. The result of our study has been to show:

1. That the neuroporic recess is situated just above the anterior commissure and below the anterior pallial commissure and between the pillars of the fornix when those structures are present. It is the recessus triangularis of Schwalbe and recessus inferior of Elliot Smith.

2. The boundary line between roof structures and area parolfactoria is marked in reptiles and many mammals by a groove running rostrad from the neuroporic recess in the ventricular surface of the medial wall and by a zona limitans in the structure of the wall. The zona limitans and ventricular sulcus are both the expression of functional differentiation of adjacent centers and indicate a rearrangement of the neurones in the gray matter adjacent to the ventricle.

3. The structures dorsal to this zona limitans include the hippocampal formation proper and the septum pellucidum or its equivalent in the brains of lower mammals and reptiles. These structures are all developed out of the roof of the selachian brain, called by the writer the primordium hippocampi.

4. The structures ventral to this zona limitans include a nucleus lateralis closely related to the head of the caudate nucleus, a nucleus medialis on the medial surface, islands of Calleja and the tuberculum olfactorium.

5. The hippocampal formation gradually differentiates a cortical layer in the medio-dorsal region of the hemisphere and, as

Elliot Smith and Levi have shown, the most medial border of this gives rise to the fascia dentata. Dorsal to the fascia dentata the hippocampus folds inward, on account of pressure from the expanding general cortex and perhaps other causes. This infolding produces the hippocampal fissure, which is known in embryos by the name *fissura arcuata*. This fissure is not present in the reptiles studied by the writer.

6. The septum pellucidum in lower mammals consists of a thick mass of gray matter which imbeds the fornix system and its commissure, becomes continuous with the hippocampal formation beneath the splenium of the corpus callosum and is connected with the supracallosal hippocampus by columns of cells between the bundles of the commissures. Rostrad the septum is continuous with the indusium around the genu corporis callosi and extends forward ventral to the precallosal hippocampus to the olfactory peduncle. This septum pellucidum represents a part of the roof of the selachian forebrain (*primordium hippocampi*) which has remained in a low stage of development. In this rostral continuation of the septum pellucidum there runs a longitudinal bundle of fibers which are collected from the basal olfactory centers and reach the hippocampus either through the fimbria or by way of the stria Lancisii. Between this fimbria-bundle and the fascia dentata a sulcus appears in reptiles and mammals which is the fimbrio-dentate sulcus of human anatomy.

7. The use of the term *primordium hippocampi* in the sense which is given to it in this paper requires some comment. The term is used by me to denote that lowly organized pallial mass in the selachian brain from part of which the hippocampal formation has developed. It is also used for the homologous mass in cyclostomes where it is situated in the telencephalon medium. It is also used for the residue which is left from this mass in higher vertebrates after the hippocampal formation has been developed. This residue is the body long known as the septum pellucidum. The hippocampal formation and septum together constitute a continuous mass of gray matter which thickens the lamina supra-neuroporica and forms the bed for the hippocampal commissure and corpus callosum.

The term *primordium hippocampi* was first used by Elliot Smith ('03) to designate that portion of the reptilian and amphibian hemisphere which corresponds to the mammalian hippocampal formation. When the writer realized that the body in the brain of fishes which he had called 'epistriatum' was in reality the forerunner of the hippocampus, he adopted the name *primordium hippocampi*. At that time the writer supposed that his *primordium hippocampi* in fishes was approximately equivalent to Elliot Smith's *primordium hippocampi* in reptiles. It now appears that the *primordium hippocampi* of the writer includes the body to which Elliot Smith gave the same name plus the equivalent of the septum.

The most important matter is that the exact meaning of terms be understood. Upon the lesser question as to what terms are most appropriate a few words may be said. Elliot Smith's *primordium hippocampi* is the equivalent of the hippocampus and *fascia dentata*. In the reptilian brain it is bounded by a sulcus which he called *sulcus limitans hippocampi*. This name is literally appropriate and it is now clear that the sulcus in selachians to which the writer applied the same name is an entirely different sulcus. The *sulcus limitans hippocampi* of the frog as recognized by Herrick and the writer is the same as that in reptiles. It is shown in this paper that this sulcus lies between the *fascia dentata* and the *fimbria*. Since the term *sulcus fimbriodentatus* is in common use and clearly understood in descriptions of the human brain, it can be used for this sulcus in reptiles and amphibians and the term *sulcus limitans* becomes unnecessary. There is, however, need for a term to designate the boundary between the pallial and sub-pallial areas in the medial wall. This is what Elliot Smith attempted to do by his term *sulcus limitans*. It is to this sulcus which limits the pallium that the writer has applied the name *sulcus limitans hippocampi*. This term is inappropriate because of the extreme divergence in structure between the hippocampus and septum in mammals, although they are indistinguishable in selachians, and remain similar in function. What is needed is some term to indicate that the septum belongs to the pallial area. This suggests such terms as *sulcus marginalis pallii* or

fovea limbica medialis, or sulcus rhinalis medialis. Since the term sulcus rhinalis (lateralis) is used for the sulcus which marks the boundary between the pallium and the olfactory area laterally, the term sulcus rhinalis medialis seems peculiarly appropriate for this sulcus in the medial wall. In other words, the writer having used the term sulcus limitans hippocampi throughout this paper in the same sense as in former papers, would now substitute for it the term sulcus rhinalis medialis. The term sulcus limitans hippocampi of Elliot Smith is synonymous with the term sulcus fimbrio-dentatus.

As for the term primordium hippocampi, the writer believes that it is best to retain this to include the equivalent of hippocampal formation plus septum pellucidum. Two considerations strongly support this. The one is that the two are undivided and indistinguishable in fishes, the other is that the fimbria is usually considered to be an integral part of the hippocampal formation but is separated from Elliot Smith's primordium hippocampi by his sulcus limitans.

8. The hippocampal commissure and corpus callosum correspond in all their morphological relations to the two commissures in the roof of the telencephalon in selachians which are related respectively to the primordium hippocampi and the somatic sensory area. The marsupial *Didelphys* possesses true corpus callosum fibers running in the dorsal forebrain commissure. This is apparently the typical condition in selachians, marsupials and mammals. A corpus callosum has not been certainly demonstrated in reptiles.

9. The development by Elliot Smith of the idea that the pallial commissures are originally imbedded in the sub-pallial paraterminal body and that the great development and arching up of the corpus callosum has stretched and raised up the paraterminal body to form the septum pellucidum, has led to the recognition of a large body situated above the foramen of Monro which, although apparently in a pallial position, has had a sub-pallial origin. This is the body known in the work of recent writers as the supraforaminal portion of the paraterminal body. This body in lizards not only forms a bed for the anterior pallial com-

missure but extends back in the medial wall of the hemisphere beneath the hippocampus to the posterior pole, where it forms the bed for the posterior pallial commissure also (Elliot Smith, '03, Herrick '10).

In the light of the whole content of the present paper it is obvious that the writer would regard the supraforaminal mass in question as not belonging to the paraterminal body at all but to the pallium. It is without question derived from the pallium of the selachian brain and is related to the lamina supraneuroporica and not to the lamina terminalis. The term paraterminal body should be restricted to the basal olfactory centers in the medial wall which are in relation with the lamina terminalis. This body never reaches above the foramen to any significant extent. The writer has recognized a small projection of the paraterminal body above the foramen in selachians but it is of no importance. Nearly the same condition exists in the mole.

The general relations of the gray masses below the neuroporic recess and the zona limitans are clear. Although we by no means understand all the factors which have called forth special collections of neurones in this region, we may say that they all belong to the medial portion of the olfactory lobe or the medial olfactory area. The tuberculum olfactorium is a basal nucleus related at its two borders with the lateral and medial olfactory nuclei. It consists of a deeper, more compact, layer containing islands of Calleja and of a superficial layer of loosely scattered cells. Both these layers are continued into the medial wall where the superficial cells form a broad thin plate on the medial surface. Between the tuberculum and the ventricle is the massive head of the caudate nucleus and this extends around the ventral angle of the ventricle to form the deep layer of the medial wall. There are, therefore, superficial, middle and deep layers of cells in the medial wall. The several cell aggregates have been designated by various authors by such names as nucleus septi, nucleus acumbens septi, nucleus medianus septi, and so forth.

The term 'septum' has been applied to two independent structures in the telencephalon, the septum pellucidum of higher mammals and the medial olfactory nucleus or area in the medial wall

of the brain of reptiles and amphibians. It has been the belief of most authors that this so-called septum in amphibians and reptiles is the equivalent of or is intimately associated with the septum pellucidum of mammals. In reality it includes both the medial olfactory nucleus and the equivalent of the septum pellucidum of mammals. Adolf Meyer ('92) and Unger ('06) distinguished the septum pellucidum as an independent structure in reptiles. The terms 'nucleus septi,' 'nucleus lateralis septi,' and so forth, are confusing and require revision. The nucleus septi (Unger; nucleus accumbens septi, Kappers) is said by these authors to belong to the striatum and not to the septum. It is divided by Herrick (fig. 43) into nucleus accumbens septi and nucleus lateralis septi. The latter nucleus includes in addition a great part of the equivalent of the septum pellucidum (figs. 47, 61, 56, 64, 66). The term 'nucleus medianus septi' is used by Herrick for part of the primordium hippocampi (figs. 43, 61), and also for the superficial layer of cells in the area parolfactoria (fig. 66). In other words, the lateral and medial nuclei 'of the septum' each contains a part of the parolfactory and a part of the pallial areas. Similar inconsistencies appear in the work of other authors. Generally speaking, recent authors have followed Elliot Smith in regarding the septum of mammals as a derivative of the paraterminal body which is basal. They have consequently applied the term septum to the basal part of the medial wall in reptiles and amphibians. In this way the term septum has been made to include both basal olfactory and pallial areas. For this reason all reference to the septum should be eliminated from the names hereafter used for the several nuclei belonging to the basal olfactory areas. With this in view I have adopted the term, area parolfactoria, and have called the superficial layer of cells the nucleus parolfactorius medialis and the deep layer which is so closely related to the caudate, the nucleus parolfactorius lateralis. It should be noted that this area corresponds nearly to the area parolfactoria of Broca, and that the writer regards as very unfortunate the recent use of the term to designate the tuberculum olfactorium or an adjacent part of the anterior perforated space (lobus parolfactorius, Edniger '08, p. 260; eminenzza paraolfat-

toria, Beccari '11). A discussion of the relations and terminology of the region surrounding the tuberculum olfactorium must be reserved for another time.

The general results of the foregoing study should be summarized lest the interest in various details should overshadow the main significance of such a comparative study. The reptilian and mammalian equivalents of the pallial and basal areas of the selachian telencephalon and the boundary between them have been accurately defined so far as the medial wall is concerned. It has been shown that the septum pellucidum or its equivalent is a derivative not of the basal olfactory area but of the pallial area. It is the unchanged residuum of the selachian pallium after the hippocampal formation is developed. The fusion of the two septa (hippocampal primordia) in the lamina supraneuroporica forms the bed for the passage of the pallial commissures between the hemispheres. It is shown that the two commissures in the selachian pallium hold essentially the same relations to all structures in the median region as are held by the hippocampal commissure and corpus callosum in mammals. It is therefore strongly probable that the commissure of the somatic area in the selachian telencephalon is the true forerunner of the mammalian corpus callosum. The final determination whether this is true or not will come with the further study of the history of the somatic area and the clear demonstration of corpus callosum fibers in reptiles and dipnoi.

The writer wishes to correct here, with due apology to the authors concerned, an unfortunate slip made in an earlier publication. In the paper on the selachian brain ('11 a, p. 58, sec. 40) occurs a sentence which should read as follows: "The terms neopallium (Elliot Smith) and archipallium (Edinger) are therefore not appropriate." I was acquainted with Professor Smith's disclaimer of the term archipallium (*Anat. Anz.*, Bd. 35, p. 429) and it was my express intention in the sentence quoted to credit the two terms to their proper authors. Through some inexplicable error the names became interchanged and the mistake was not noticed until recently.

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REFERENCE LETTERS

- a.h.*, association bundles of the hippocampus
alv., alveus
am., amnion
a.t., angulus terminalis
b.o., bulbus olfactorius
c., chorda dorsalis
c.a., commissura anterior; also the commissural bundle of the olfactory tract
cbl., cerebellum
c.c., corpus callosum
c.d., cortex dorsalis
c.e., capsula externa
c.f., columna fonicis
cg., cingulum
c.h., commissura hippocampi
c.i., capula interna
ch.op., chiasma opticum
c.l., cortex lateralis
c.m., corpus mammillare
c.post., commissura posterior
c.p.a., commissura pallii anterior
c.p.p., commissura pallii posterior
c.r., corona radiata
c.s., commissura superior
c.st., corpus striatum
dec.po., decussatio postoptica
dienc., diencephalon
e., epiphysis, pineal body
em.th., eminentia thalami
ep., epistriatum
f., fornix
fasc.m., fasciculus marginalis
f.pc., fasciculus praecommissuralis
f.d., fascia dentata
f.sag., fissura sagittalis
fi., fimbria
flex.h., hippocampal flexure
f.o., formatio olfactoria
for.i., foramen interventriculare
f.rh., fissura rhinalis
f.s., fornix superior
g., genu corporis callosi
h., hippocampus
hab., nucleus habenulae
hem., hemisphere
hy., hypothalamus
hyp., hypophysis
i., indusium
i.C., islands of Calleja
l.pyr., lobus pyriformis
l.s., lamina supraneuroporica
l.t., lamina terminalis
m., margo posterior pallii, caudal margin of the lamina supraneuroporica
n., neuropore
n.c., nucleus caudatus
n.m., nodulus marginalis
n.o.a., nucleus olfactorius anterior
n.p.l., nucleus parolfactorius lateralis
n.p.m., nucleus parolfactorius medialis
n.t., nervus terminalis
n.tr.olf.lat., nucleus of the lateral olfactory tract
p., paraphysis
para., paraterminal body
p.f., perforating fibers of fornix superior
p.h., primordium hippocampi
r.c.c., rostrum corporis callosi
r.i., recessus infundibuli
r.p., recessus praeopticus
r.po., recessus postopticus
r.m., recessus mammillaris
r.n., recessus neuroporicus
r.n.e., recessus neuroporicus externus
r.s., recessus superior
s.c.c., sulcus corporis callosi
s.d., saccus dorsalis
s.en., sulcus endorhinalis
s.f-d., sulcus fimbrio-dentatus
s.hy., sulcus hypothalamicus (sulcus Monroi)
s.l., sulcus limitans hippocampi
s.l.H., sulcus limitans of His
s.l.L., stria lateralis Lancisii
s.m., stria medullaris
s.m.L., stria medialis Lancisii
s.o., sulcus olfactorius

spl., splenium
s.t., stria terminalis
sub., subiculum cornu ammonis
t.c., tela chorioidea
t.f., taenia fornicis
thal., thalamus
th.r., thalamic radiations

t.o., tuberculum olfactorium
t.p., tuberculum posterius
tr.olf., tractus olfactorius
tr.op., tractus opticus
v.l., ventriculus lateralis
v.tr., velum transversum
z.l. zona limitans

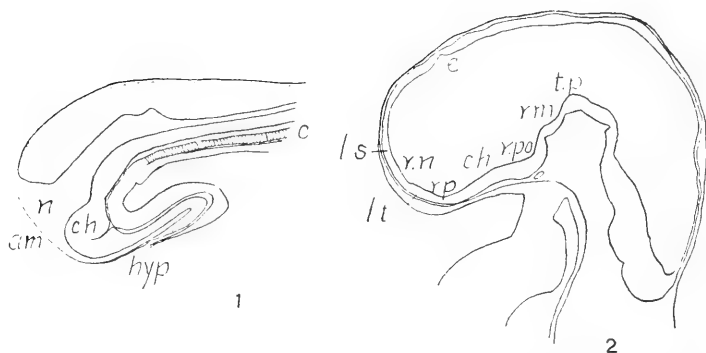
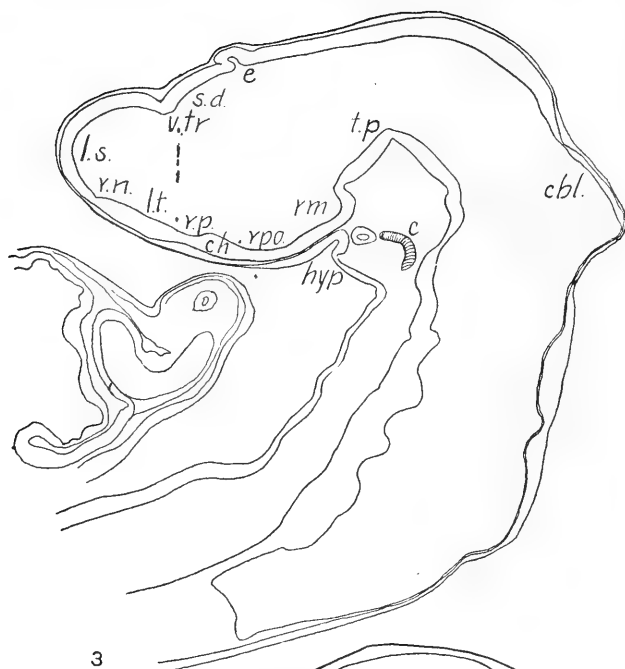
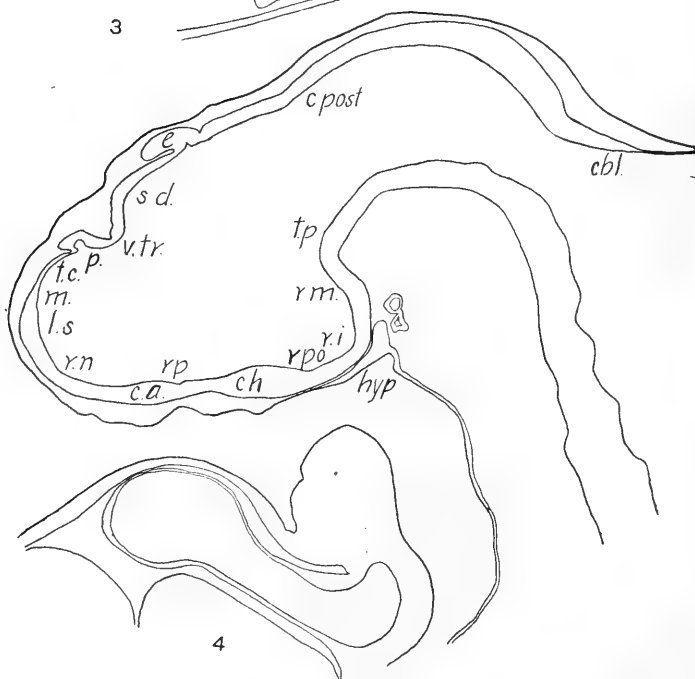


Fig. 1 *Chelydra serpentina*, 2 mm. embryo, median sagittal section of the anterior portion to show the neuropore and adjacent structures. Magn. 52 diam.

Fig. 2 *Chelydra serpentina*, 4.5 mm. embryo, sagittal section of head to show neuroporic recess, lamina terminalis and lamina supraneuroporica. Magn. 52 diam. The section is median in the anterior region.



3



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Fig. 3 *Chelydra serpentina*, 6 mm. embryo, median sagittal section. Magn. 52 diam.

Fig. 4 *Chelydra serpentina*, 9 mm. embryo, median sagittal section. Magn. 52 diam.

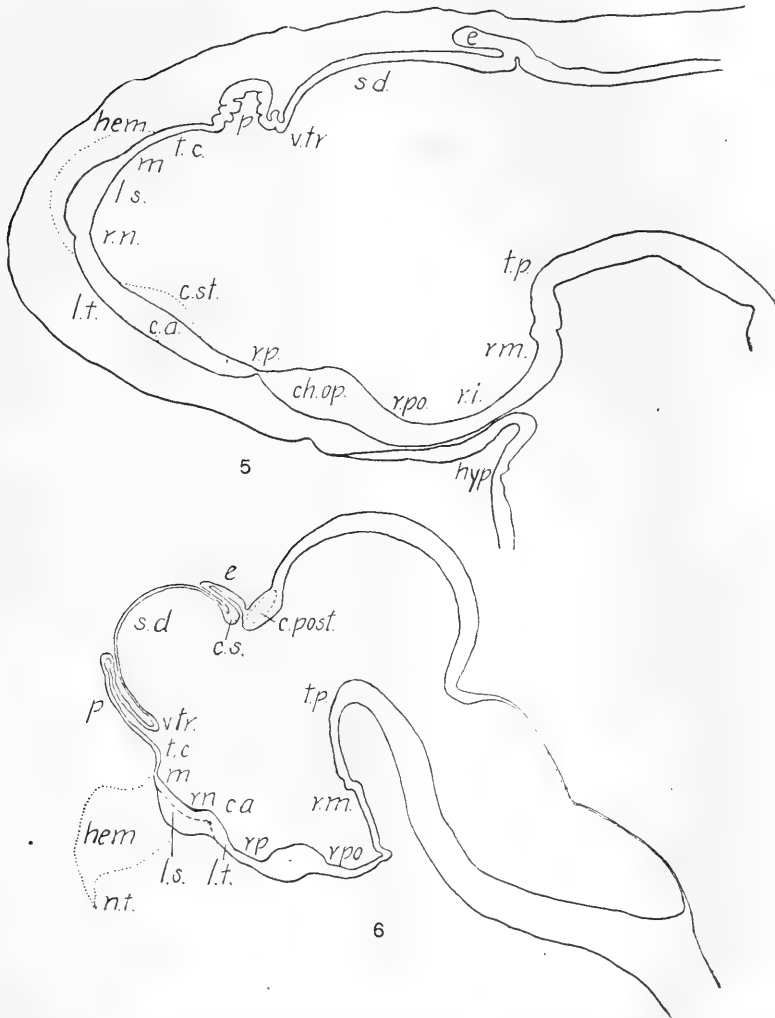


Fig. 5 *Chelydra serpentina*, 10 mm. embryo, median sagittal section. Magn. 52 diam. In the 6, 9 and 10 mm. stages the lamina supraneuroporica grows in thickness. The point of transition to the tela is the margo posterior pallii. The fibers of the anterior commissure are evident in this stage.

Fig. 6 *Chelydra serpentina*, median sagittal section of the brain of a specimen having a carapace 8 mm. in length. Magn. 17 diam. The thickening of the lamina supraneuroporica now begins to encroach upon the recessus neuroporicus, which is nearly obliterated in the adult.

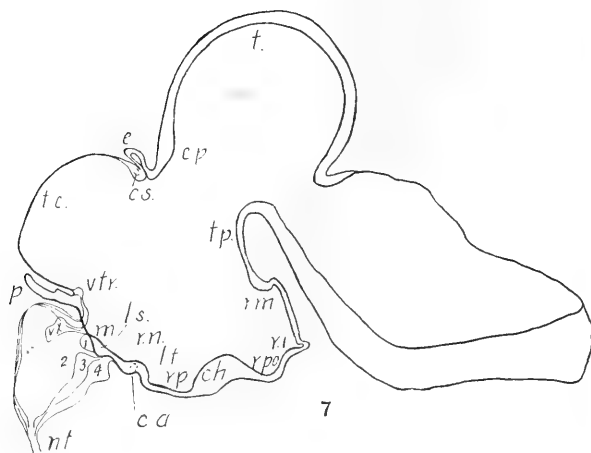


Fig. 7 *Emys lutaria*, median sagittal section of the brain of an embryo slightly more advanced than that shown in figure 6.

Fig. 8 Human embryo, 31 mm. C. R. length, median sagittal section of brain. Huber collection, No. XLVII. In the relations about the neuroporic recess this embryo agrees essentially with the turtle embryos.

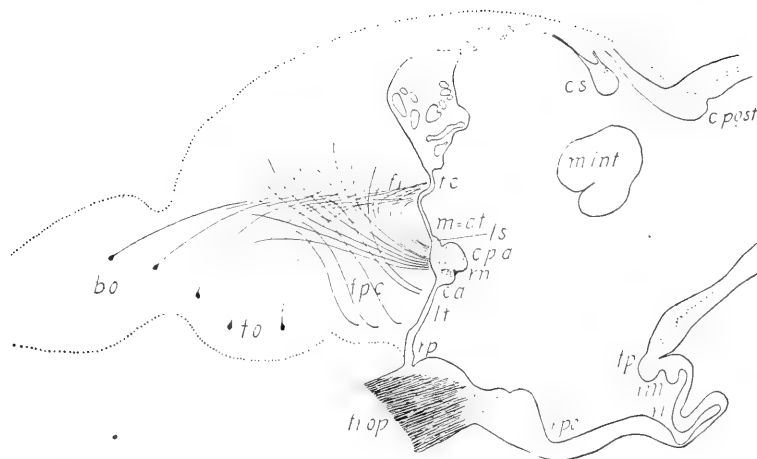


Fig. 9 *Cistudo carolina*, adult, median sagittal section of forebrain. The pallial commissure has descended into close relation with the anterior commissure so that the neuroporic recess is nearly obliterated. The median section is accurately reconstructed from sagittal sections. The dotted outline of the hemisphere is diagrammatic. For the explanation of the fibers shown, see text.

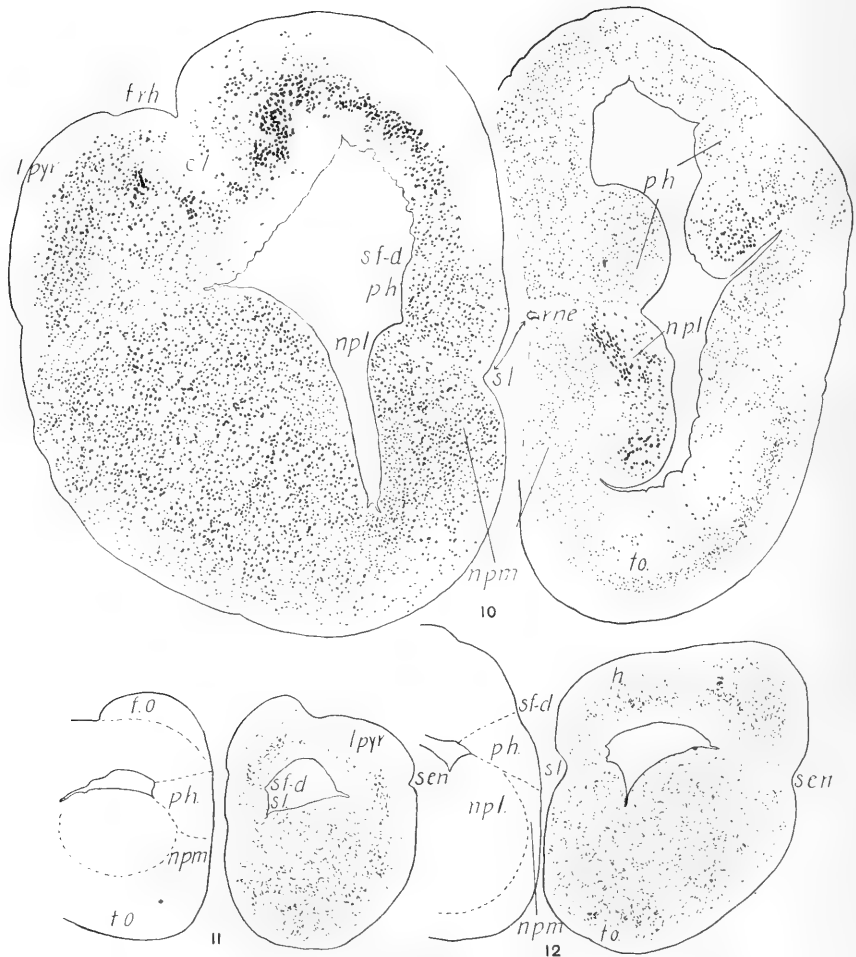
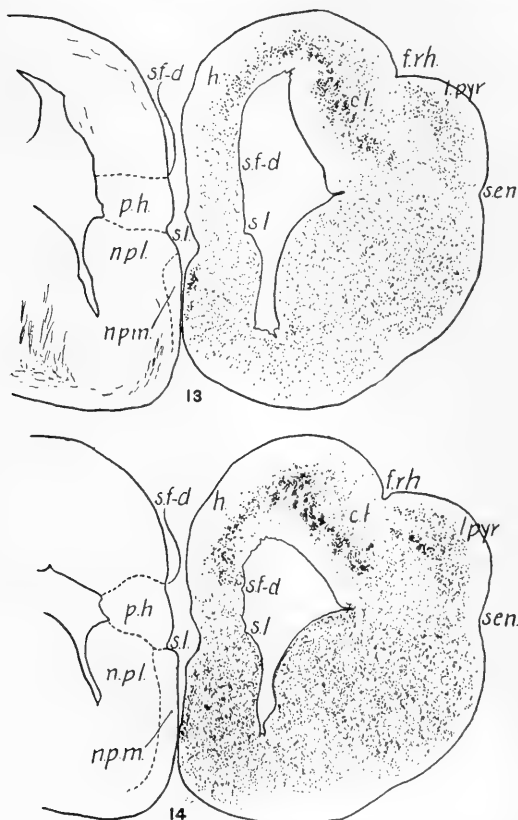


Fig. 10 A figure for the comparison of the selachian brain with that of the turtle. The right half of the figure represents a section of the brain of *Acanthias* (from '11 a, fig. 75). The left half represents a section at a corresponding level of the brain of the turtle. Both drawings were made under the Edinger apparatus and the grouping of cells is accurately represented. At this level in the brain of *Acanthias* the nucleus parolfactorius lateralis appears to be confined to the medial wall, but farther rostrad it has a broad connection with the deep gray of the lateral wall quite as in mammals. It does not appear that this nucleus represents an invasion of the medial walls by the striatum. It is rather a primary component of the medial wall.



Figs. 11 to 18 *Cistudo carolina*. Eight transverse sections of the hemispheres. The right half of each section is drawn from a series stained with a cell stain, the left half from a Weigert series. Owing to variation between individuals and to slight differences in the plane of section the outlines of the two halves do not agree perfectly, but the right and left sides are substantially equivalent. The drawings were made under the Edinger apparatus. On the right side each cell is represented by an ink dot. The number and grouping of the cells are accurately shown, the relative size fairly well. Magn. 13 diam.

Fig. 11 Section through the olfactory peduncle and the caudal border of the formatio olfactoria. It shows the two ventricular sulci separating from one another in front of the peduncle (compare fig. 19). Between the two is the primordium hippocampi and above this the rostral end of the hippocampus.

Fig. 12 Section in the caudal part of the peduncle showing the two ventricular sulci close together.

Figs. 13 and 14 Successive sections farther caudad. The sulcus limitans between the area parolfactoria and the primordium is better marked than that (*s. f.-d.*) between the primordium and hippocampus.

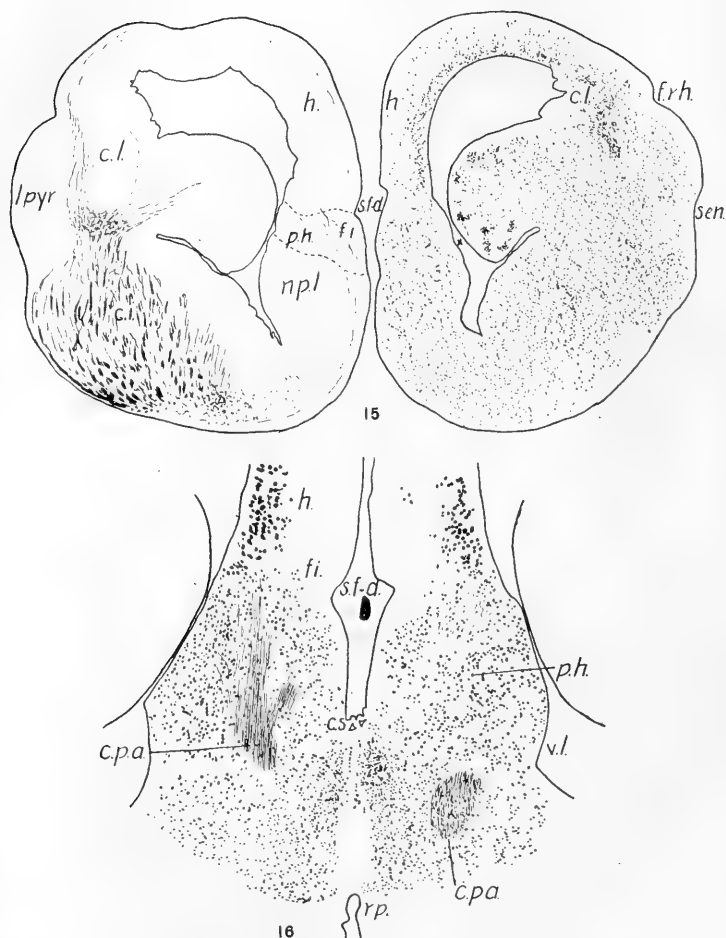


Fig. 15 Section at about the level where the sulcus limitans bends downward and is less conspicuous in transverse sections. It is marked by a broken line on the left and on the right by a small cross.

Fig. 16 A section taken just at the rostral border of the superior recess. The section is magnified 28 diameters. The anterior pallial commissure rises at either side in the hippocampal primordium. The two dense nuclei of small cells near the middle line are in the rostral wall of the superior recess and correspond in part to the nodulus marginalis of the bat and rodents. A part of this nucleus is represented also by the small-celled nucleus adjacent to the hippocampal commissure (e.g., in figs. 46 and 64). c. s., recessus superior.

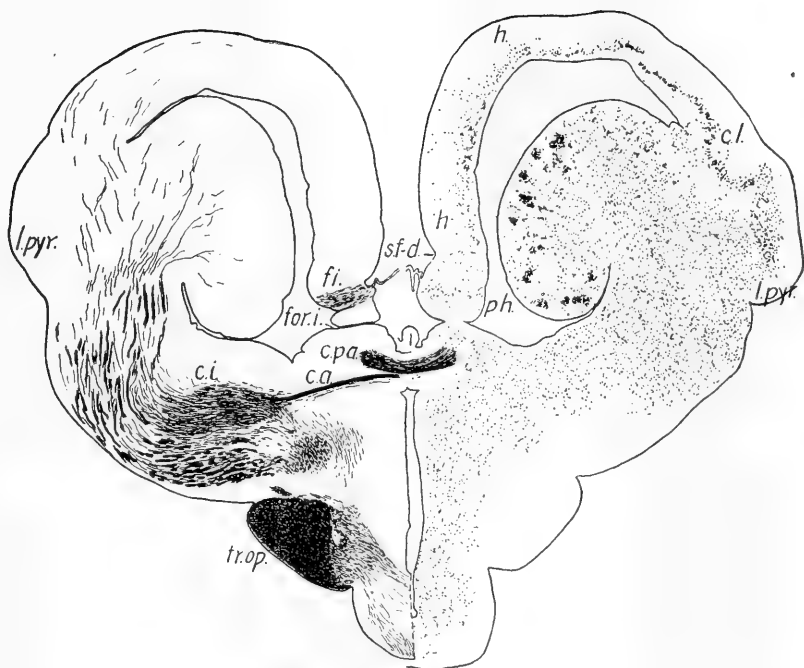


Fig. 17 Section at the rostral border of the interventricular foramen passing through the commissures. At this level the hippocampal primordium rapidly decreases in size, as seen on the left.

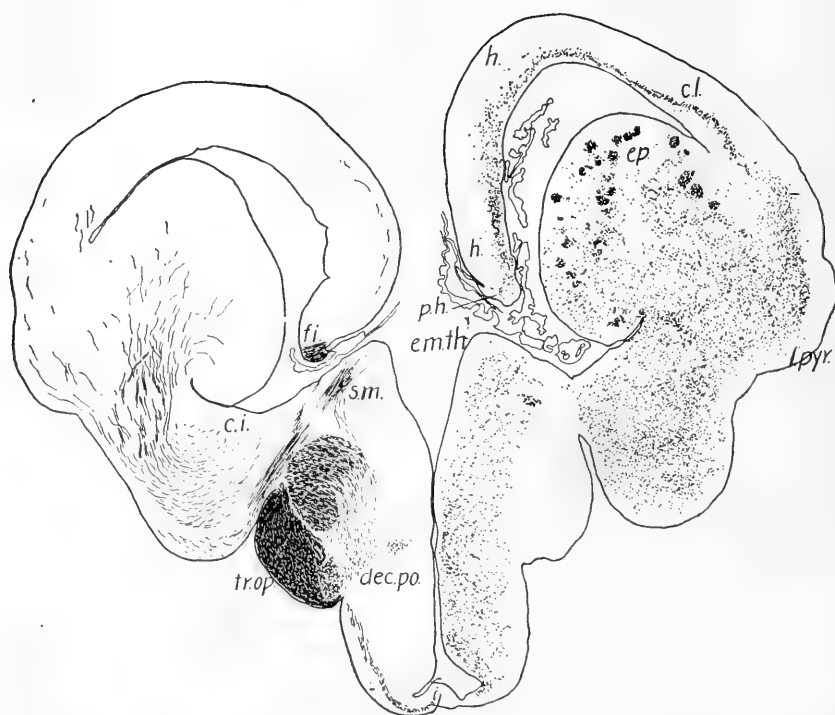


Fig. 18 Section near the caudal border of the foramen. The primordium is small here and is nearly filled by the fibers of the fimbria.



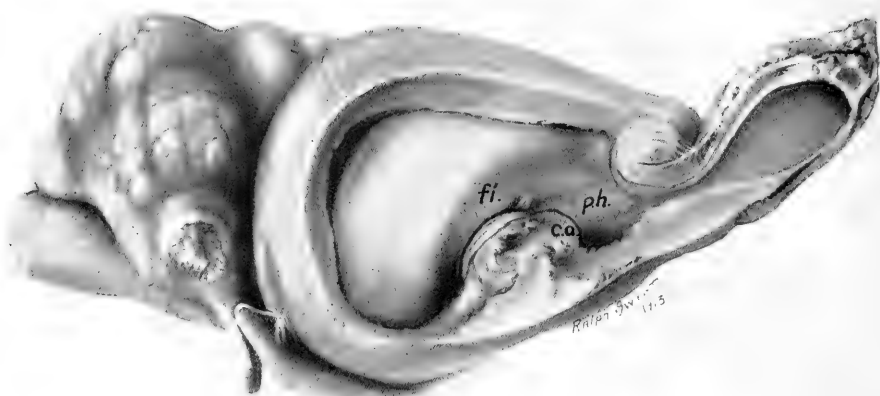
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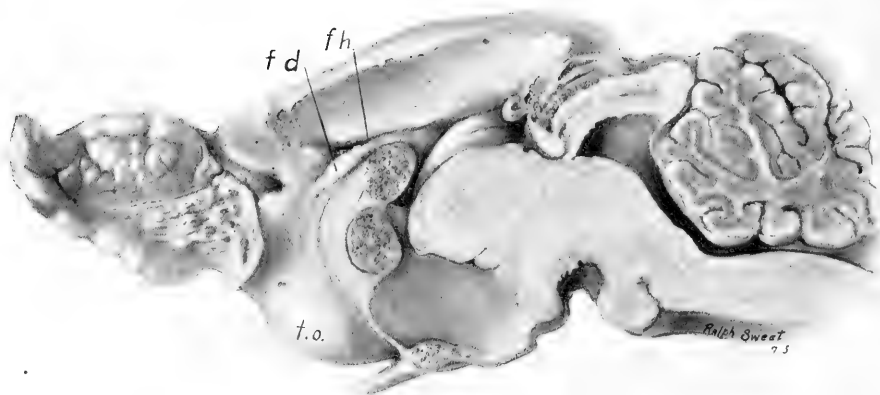
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Fig. 19 *Cistudo carolina*, dissection of the right hemisphere to show the medial wall through the lateral ventricle. Nearly the whole lateral wall of the hemisphere and the choroid plexus have been removed. The rough surface in the lower part represents the region in which the large fiber bundles were dissected away by means of needles. The area parolfactoria appears as a somewhat bean-shaped ridge. Between this and the foramen is the greater part of the primordium hippocampi. It extends forward as a very slender ridge in the groove over the area parolfactoria and becomes enlarged in the olfactory bulb. Compare figures 18 and 19. The light ridge over the primordium is occupied by the fimbria.

Fig. 20 *Cistudo carolina*, medial view of the right hemisphere after removal of the brain stem. Note especially the slender primordium hippocampi and the fimbrio-dentate sulcus extending into the temporal region of the hemisphere. In lizards the primordium is much larger.



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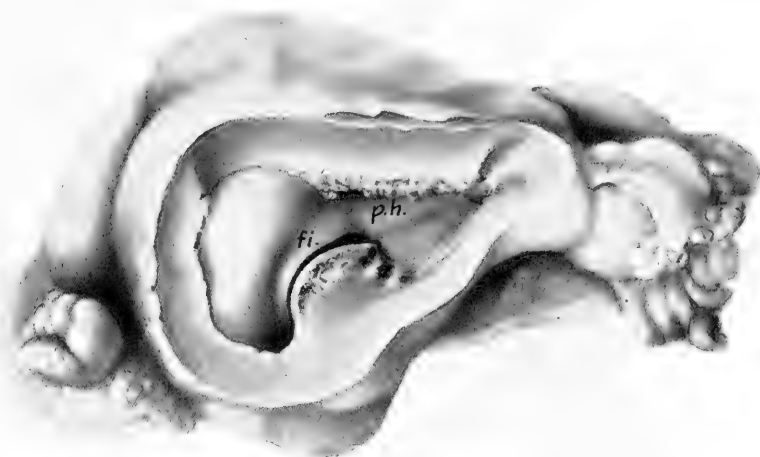


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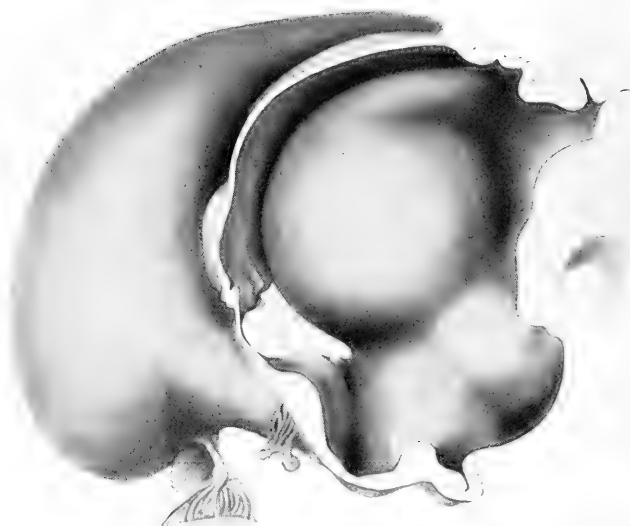
Fig. 21 Opossum (*Didelphys virginiana*). Dissection of the right hemisphere, as in figure 19. The area parolfactoria is relatively much smaller than in the turtle. The sulcus limitans is nearly horizontal in position.

Fig. 22 Opossum, medial view of the right half of the same brain as shown in figure 21. Description in the text.

Fig. 23. Striped gopher (*Spermophilus tridecemlineatus*). Dissection of right hemisphere as in figure 19. The sulcus limitans runs straight forward from the foramen. Note how the fimbria continues directly caudad from the primordium hippocampi.

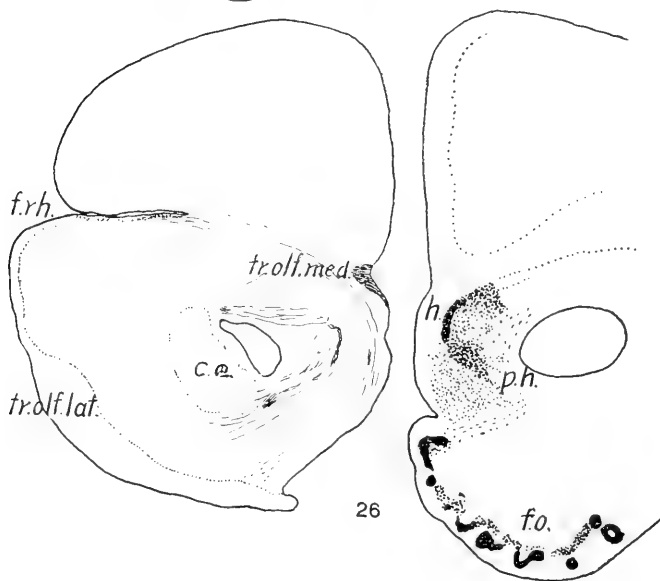
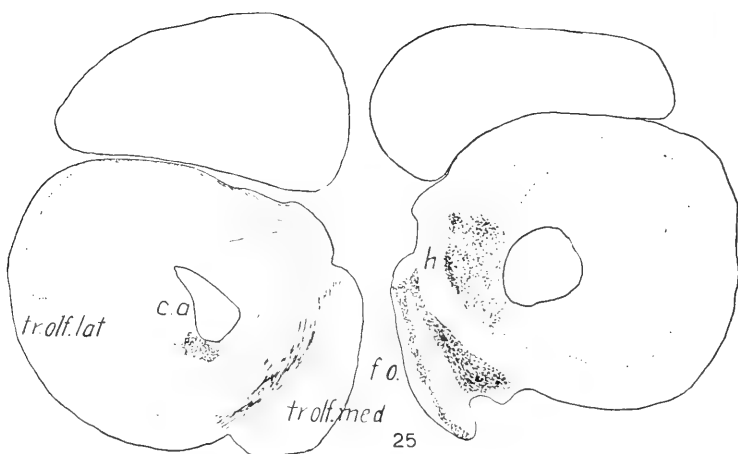


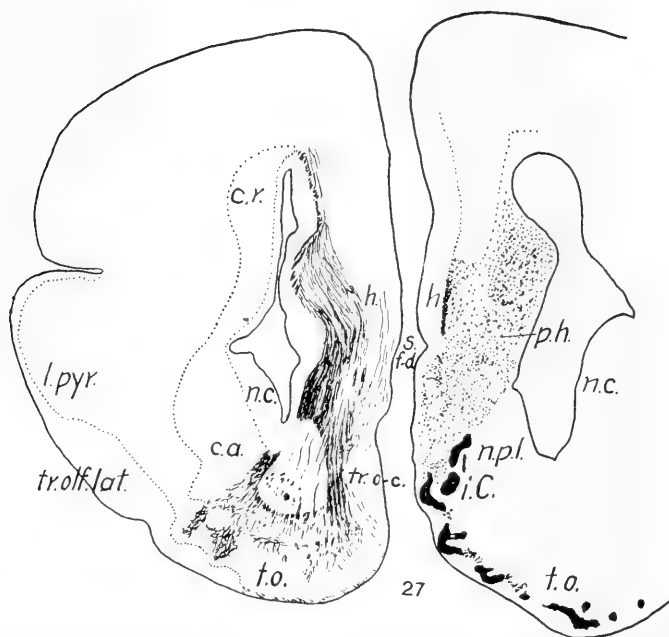
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Fig. 24 Human embryo of 31 mm. Huber collection No. XLVII. Model of the medial portion of the right half of the brain. For reference letters, see figure 8. The model was made from sagittal sections for the purpose of reconstructing the median plane and the structures about the neuroporic recess. Not enough sections were included in the model to give the complete boundary of the inter-ventricular foramen. The recessus neuroporicus is between *l.l.* and *l.s.* The blood vessels related to it are better drawn in figure 8. Note the well-marked sulcus hypothalamicus which crosses the sulcus limitans of His to form the sulcus Monroi.



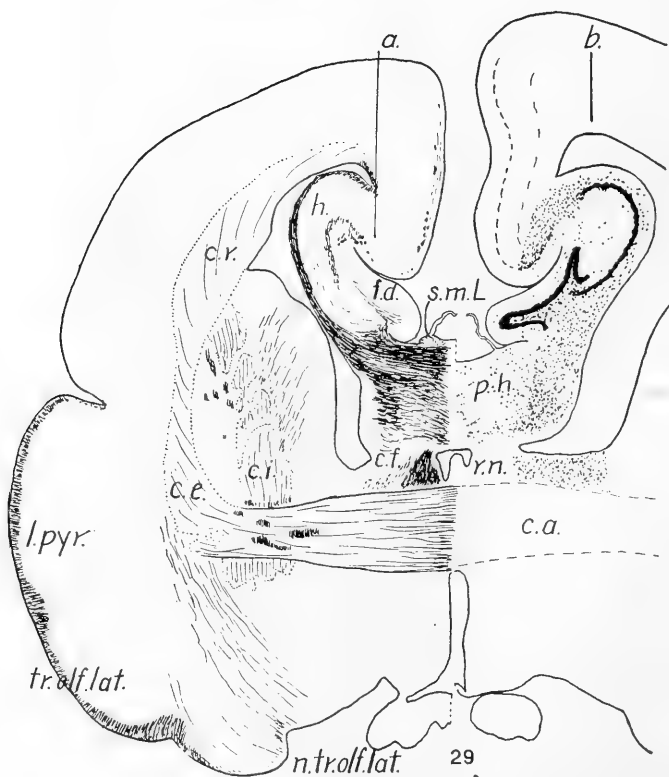
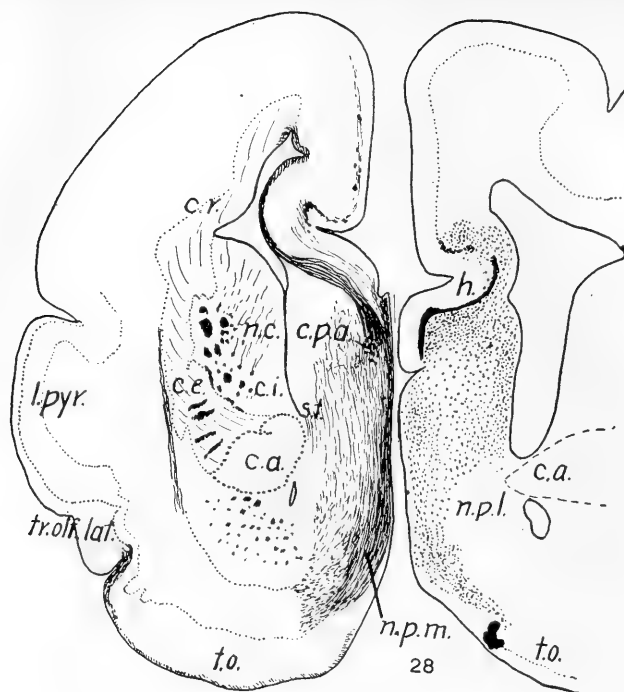


Figs. 25 to 30 Opossum, a series of transverse sections to show the relations of structures in the medial wall of the hemisphere. The right half of each figure is drawn from a series of cell-preparations, the left half from a Weigert series. On the right side the cells of the hippocampal formation and adjacent structures are drawn under the Edinger apparatus. The magnification (10 diam.) is too low to allow the form or size of the individual cells to be represented in most cases. The grouping is accurate. On the left side are drawn the fiber tracts of the medial wall.

Fig. 25 Opossum, transverse section through the olfactory peduncle. Description in the text.

Fig. 26 Opossum, transverse section just caudal to the olfactory peduncle. For the destination of the medial olfactory tract, see figure 35. Note the mass of large cells in the primordium hippocampi.

Fig. 27 Opossum, transverse section through the rostral end of the caudate nucleus and olfactory tubercle. The olfacto-cortical fibers arising from the tuberculum curve around the rostral surface of the nucleus parolfactorius lateralis, and appear to be interrupted in this section. Note the great extent of the primordium hippocampi.



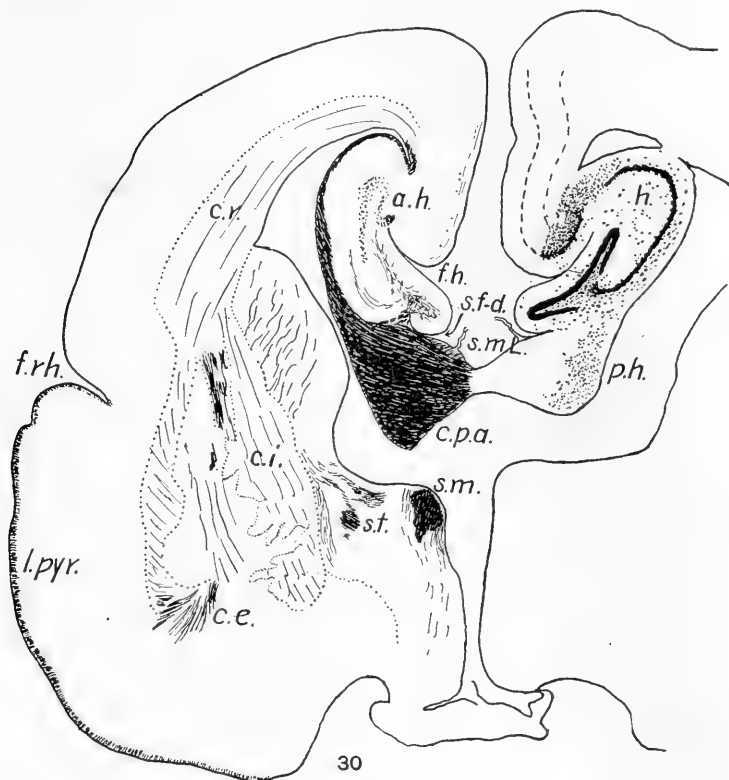
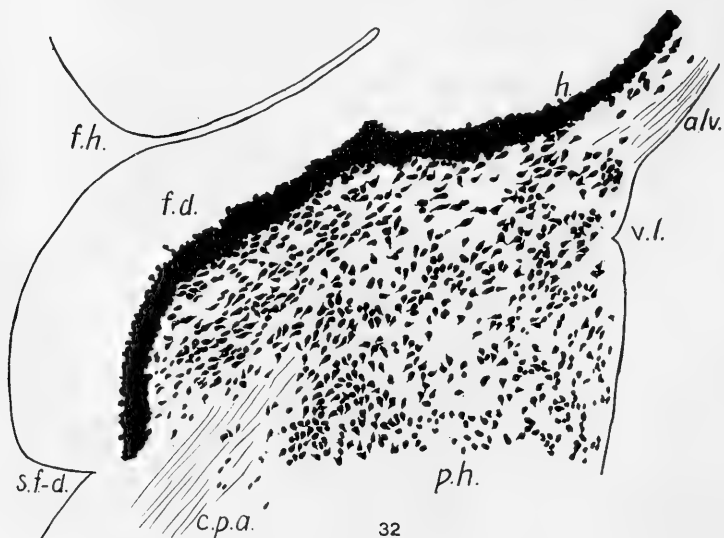


Fig. 28 Opossum, transverse section a short distance rostral to the anterior commissure. Note that the nucleus parolfactorius medialis is imbedded in the precommissural bundle. Note the continuity of the fascia dentata and the hippocampus and the close connection of the primordium with both. The rostral limb of the anterior pallial commissure contributes most of the fibers to the alveus at this level.

Fig. 29 Opossum, transverse section through the neuroporic recess. Here is seen the typical fully developed hippocampal formation with the primordium transversed by the anterior pallial commissure. All above the neuroporic recess belongs to the pallium. The commissure was thicker in the Weigert specimen than in the other. For the stria medialis Lancisii compare figure 37.

Fig. 30. Opossum, transverse section through the interventricular foramen and at the caudal border of the anterior pallial commissure. The fusion of the hippocampus with the overlying dorsal wall seen in the right half of the figure occurs frequently. In these fused places fibers seem to pass from the corona radiata into the alveus. These would probably be regarded as corpus callosum fibers. Of the three bundles constituting the stria terminalis the middle one enters the anterior commissure, the lower one passes beneath the anterior commissure, and the upper one passes over the anterior commissure and down in front of it.

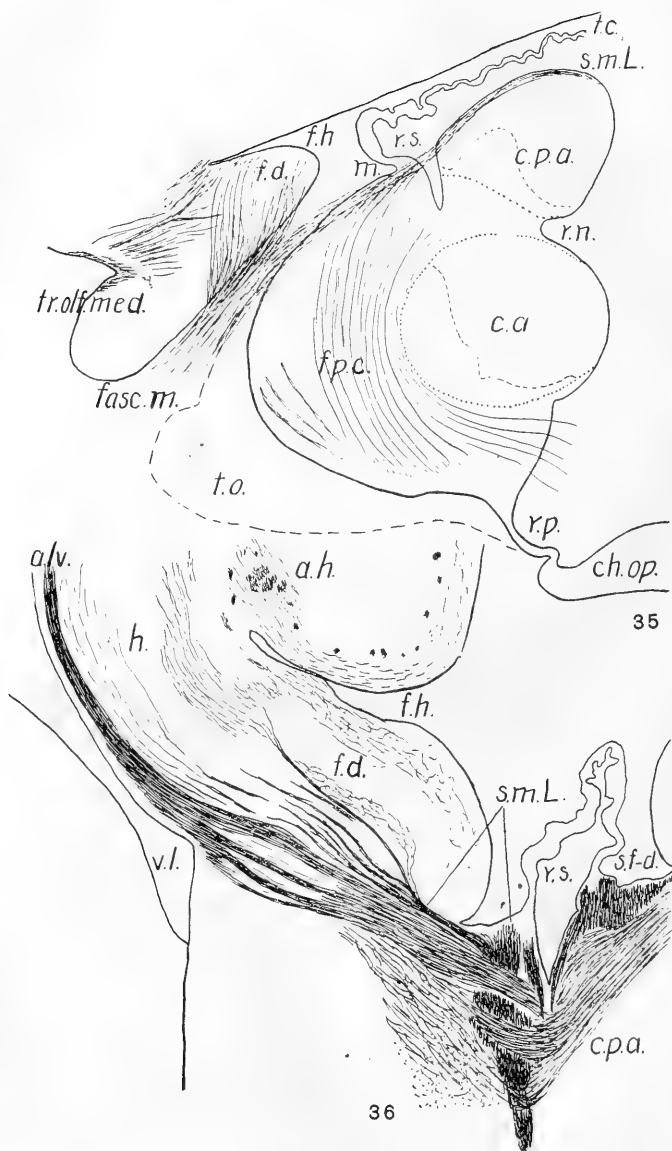




Figs. 31 and 32 Opossum, two transverse sections through the hippocampal formation to show the relations of the primordium. Cell preparation. Description in text.

Fig. 33 Opossum, part of a parasagittal section cutting the rostral limb of the anterior pallial commissure. Its position is indicated by the line *b* in figure 29.

Fig. 34 Similar figure to the last, from another specimen. Section nearer the median plane (line *a*, fig. 29). Both figures show that the commissure fibers as they pierce the wall to reach the alveus are arranged in larger and smaller bundles and that between these a considerable amount of space is filled with cells which establish continuity between the primordium hippocampi and the polymorphic layer of the hippocampus. Magn. 60 diam.



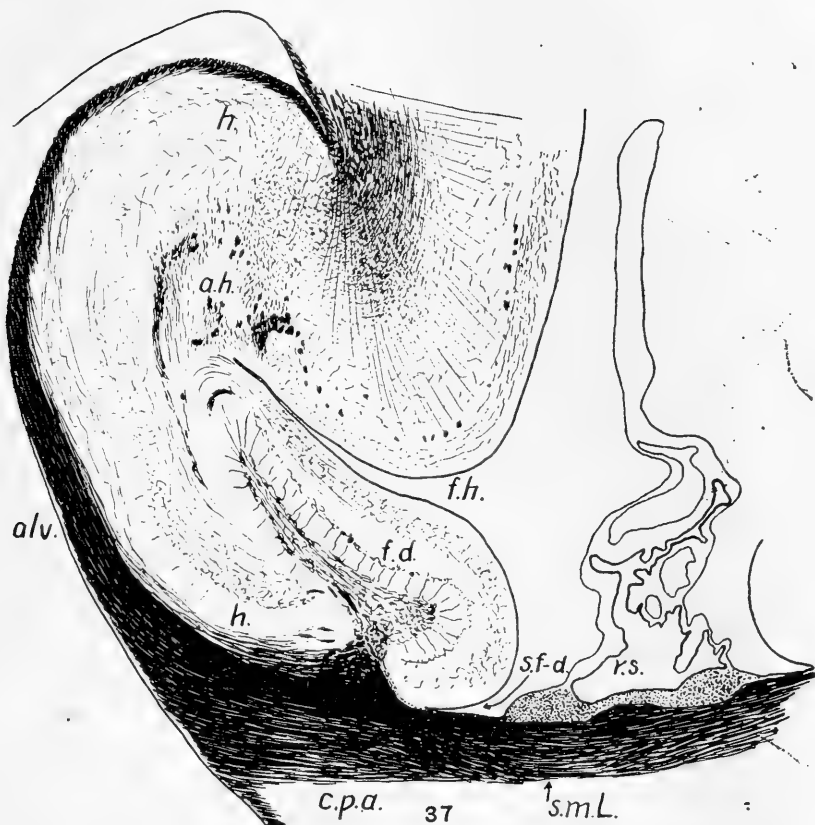
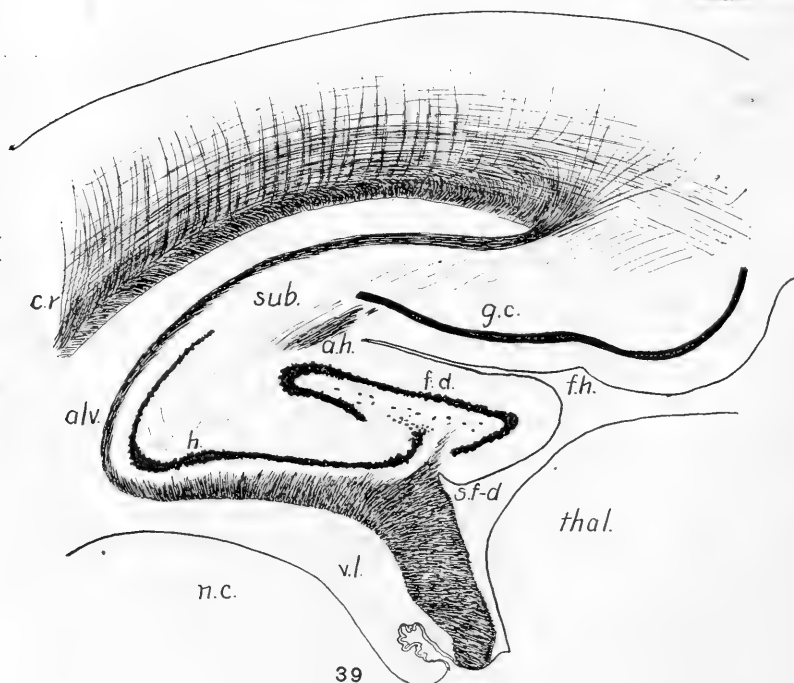
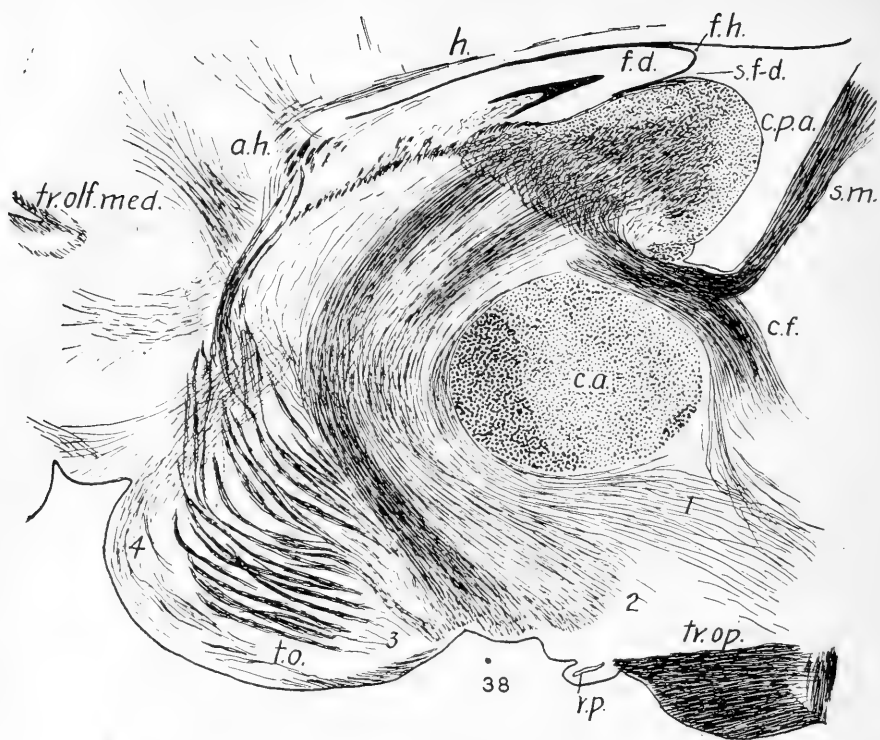
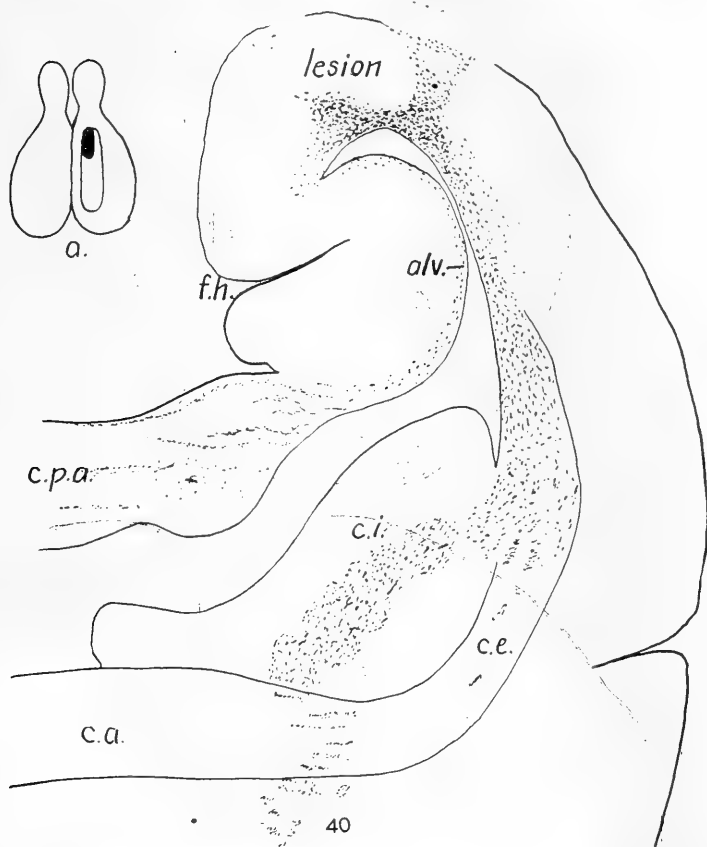


Fig. 35 Opossum, median sagittal section of the region of the cerebral commissures. Reconstructed from several sections, with addition of a few fiber bundles which lie close to the median plane.

Fig. 36. Opossum, transverse section at the rostral border of the anterior pallial commissure to show the stria medialis Lancisii coming up from the pre-commissural bundles and sending bundles to the fascia dentata and hippocampus.

Fig. 37 Opossum, from the same section as the left half of figure 29. It is evident in this section that the alveus is continuous around the dorsal angle of the ventricle with the corona radiata. The hippocampus stops at *h* and the transitional region (subiculum) between hippocampus and general cortex is bounded by the first radiating fibers. One would judge from this section that the alveus carries fibers from the general cortex to the anterior pallial commissure. Compare figures 39 and 40. The stria medialis Lancisii is a well defined bundle to which the membranous wall of the superior recess is attached.





[Fig. 38 Opossum, parasagittal section through the fornix columns and pre-commissural fibers. 1, hypothalamic fibers; 2, fibers coming from the lateral olfactory area; 3, fibers, part arising in and part passing through the olfactory tubercle. Some of these go up to enter the association bundle of the hippocampus. This may receive fibers also from the medial olfactory tract (fig. 35).

Fig. 39 Opossum, parasagittal section farther lateral than the last. The section shows especially how the alveus after passing over the hippocampus and subiculum runs on the ventricular surface of the general cortex and bends around the angle of the ventricle to be distributed to the dorsal cortex. Compare figures 37 and 40.

Fig. 40 Opossum (no. 11) section to show the degeneration of fibers following a lesion in the dorsal cortex. In the small outline at the left the area of cortex destroyed is shown by a black spot. This section is taken at the caudal end of the lesion. Small bundles in the external capsule showing degeneration are traced down to the amygdaloid region. The area outlined in the small figure is the area destroyed in the second operation described in the text.

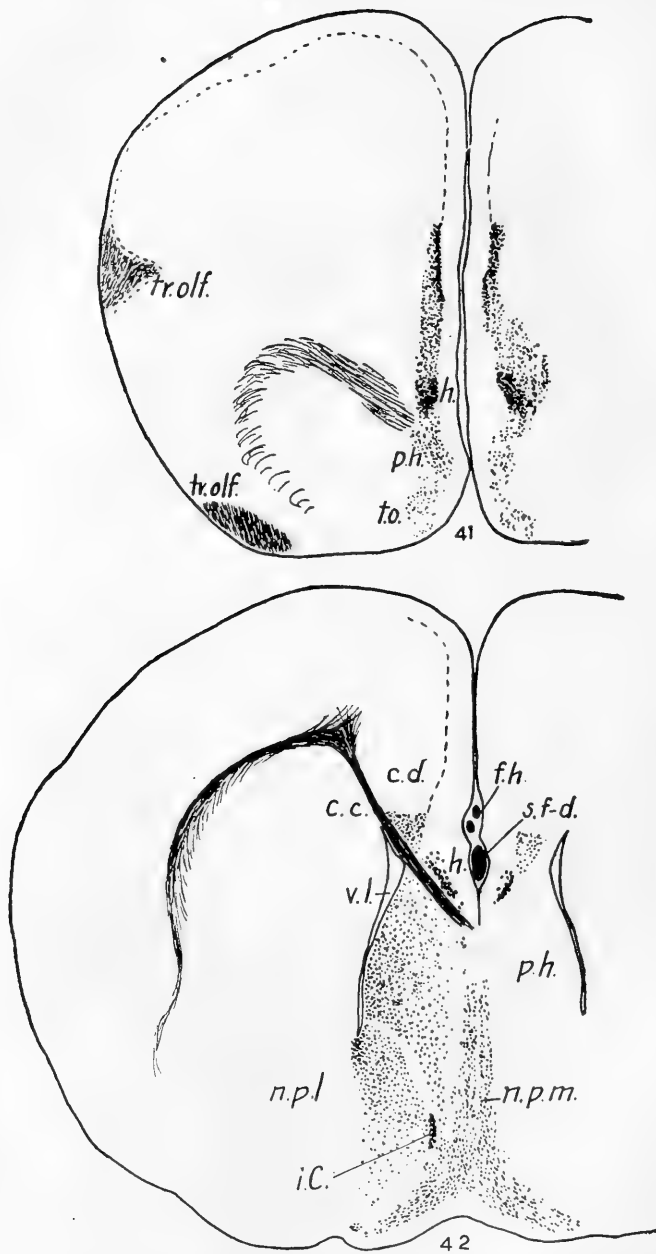


Fig. 41 Bat (*Myotis*). Transverse section near the olfactory peduncle (line *a* in fig. 45). Just enough of the cells are drawn under the Edinger apparatus to enable one to distinguish the general cortex, hippocampus, primordium hippocampi and tuberculum olfactorium. Magn. 27 diam.

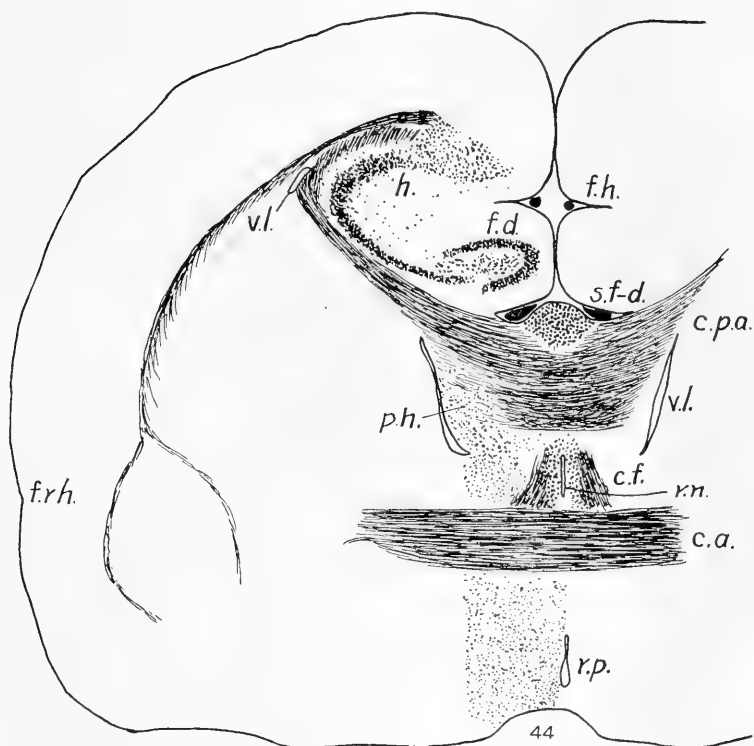
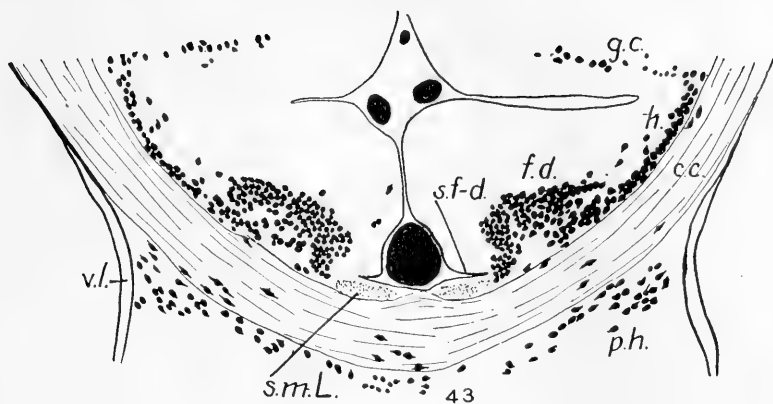


Fig. 42 Bat, transverse section through the genu (line *b* in fig. 45). The nuclei medialis and lateralis and the hippocampal primordium are especially well differentiated. Magn. 27 diam.

Fig. 43 Bat, transverse section through the middle of the horizontal limb of the anterior pallial commissure. Magn. 75 diam. It shows especially the relations of the stria medialis Lancisii.

Fig. 44 Bat, transverse section through the neuroporic recess (line *c*, in fig. 45). Description in text.

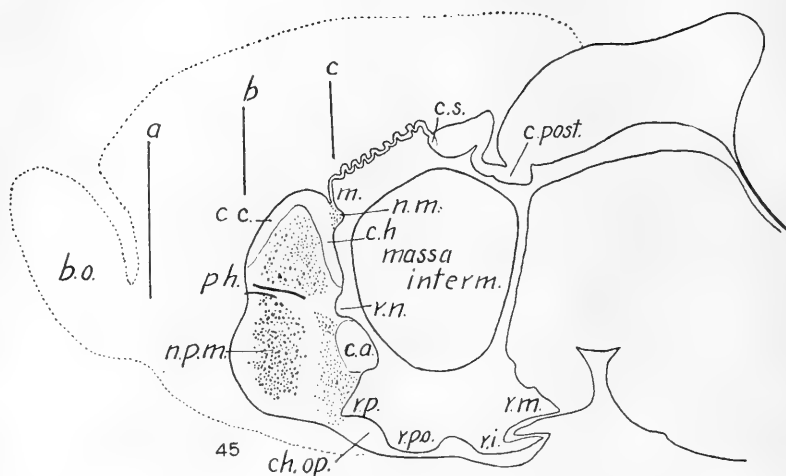
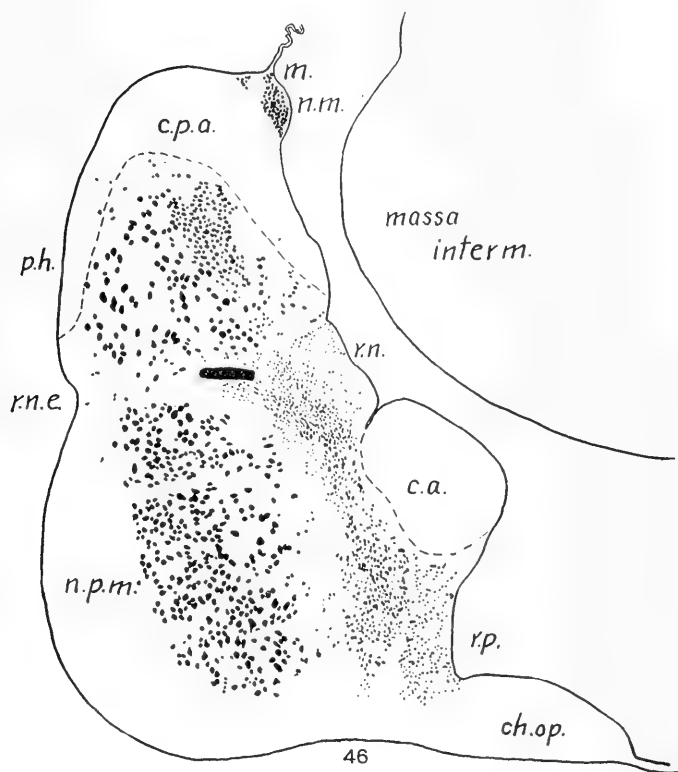
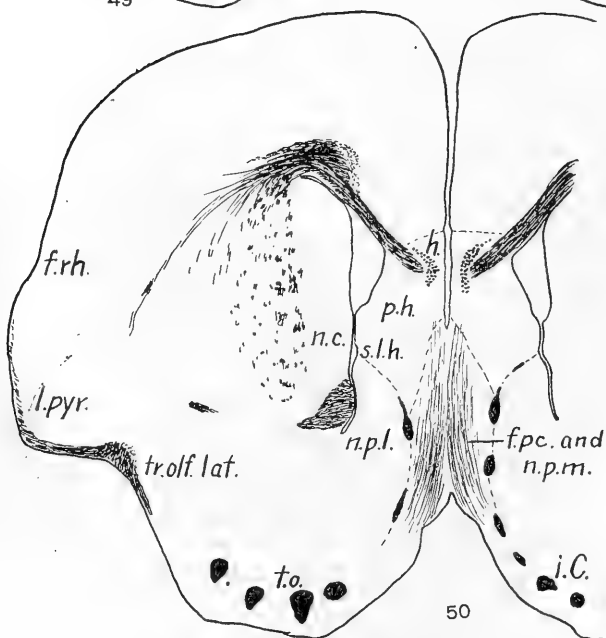
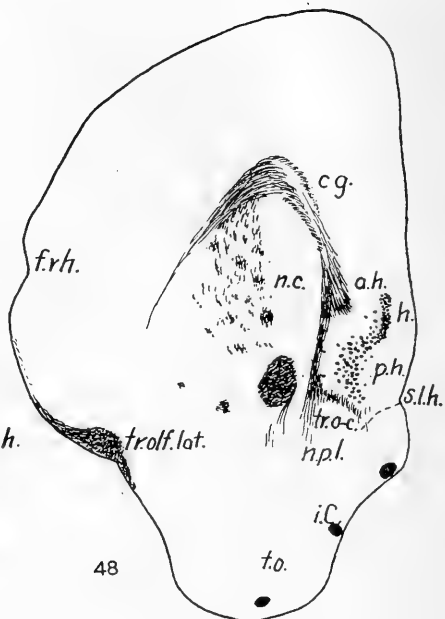
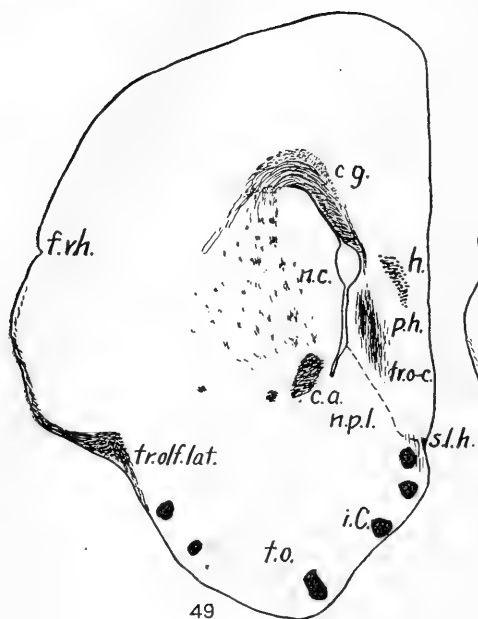


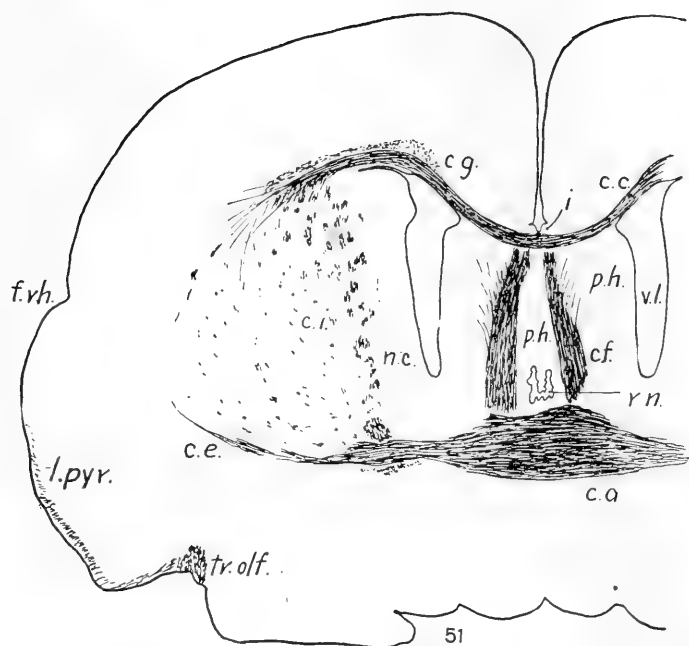
Fig. 45 Bat, median sagittal section reconstructed from several sections. Note the depth of the neuropilic recess and the two large blood vessels penetrating the brain to its vicinity. The cells are drawn free-hand. Compare figure 46.

Fig. 46 Bat, nearly median sagittal section drawn under the Edinger apparatus to show the size and arrangement of cells in the paraterminal body and the primordium hippocampi. This section being at one side of the middle plane does not show the depth of the neuropilic recess (compare fig. 45).

Fig. 47 Bat, parasagittal section to show the relations of the precallosal hippocampus. The section is drawn not far from the median plane, passing through the fornix column of one side. It shows that the anterior pallial commissure is penetrated by numerous cells of the hippocampal primordium. At *h* is the club-shaped enlargement of the pre-callosal hippocampus. Magn. 50 diam. It is strictly not correct to designate any part of the commissure complex as corpus callosum. The rostral limb contains many hippocampal fibers. It is likely that it differs from the corresponding part of the anterior pallial commissure in the opossum only in having a somewhat larger number of callosal fibers.







Figs. 48 to 53 Mole (Scalops), six transverse sections from a Weigert series. Magn. 12 diam.

Fig. 48 Mole, transverse section through the rostral end of the hippocampus (line *a* in fig. 55). Note the enlarged and in-curved primordium containing large cells. Compare with figure 26 (Opossum).

Fig. 49 Mole, transverse section between the foregoing and the genu (line *b* in fig. 55). Description in text.

Fig. 50 Mole, transverse section at genu corporis callosi (line *c* in fig. 55). The medial parolfactory nucleus is coextensive with the precommissural bundle. The nucleus is very similar to that in the bat (fig. 42).

Fig. 51 Mole, transverse section through neuroporic recess (line *d* in fig. 55). The mole is conspicuous for the large volume of primordium hippocampi which lies adjacent to the ventricle and lateral to the fornix and fimbria.

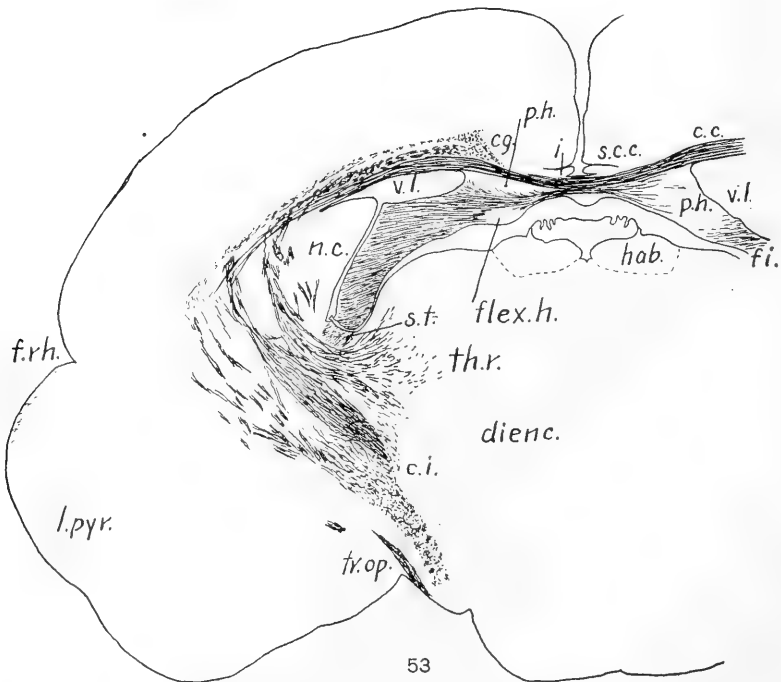
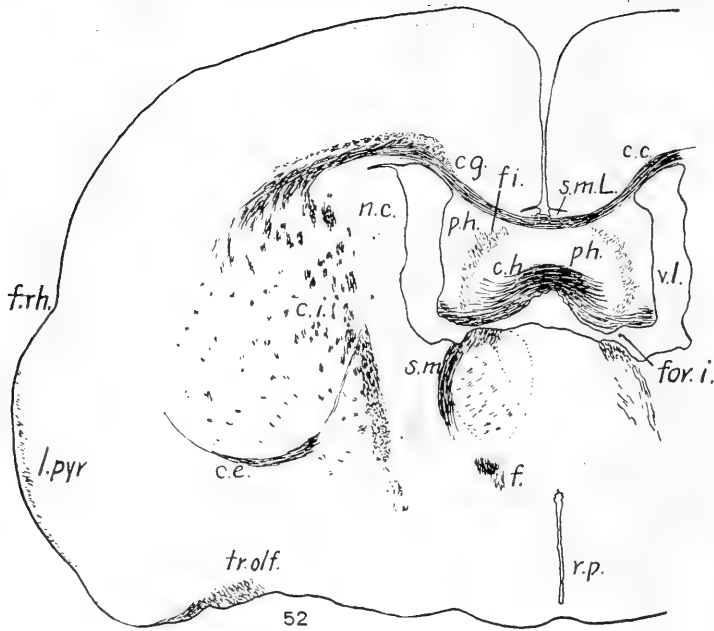
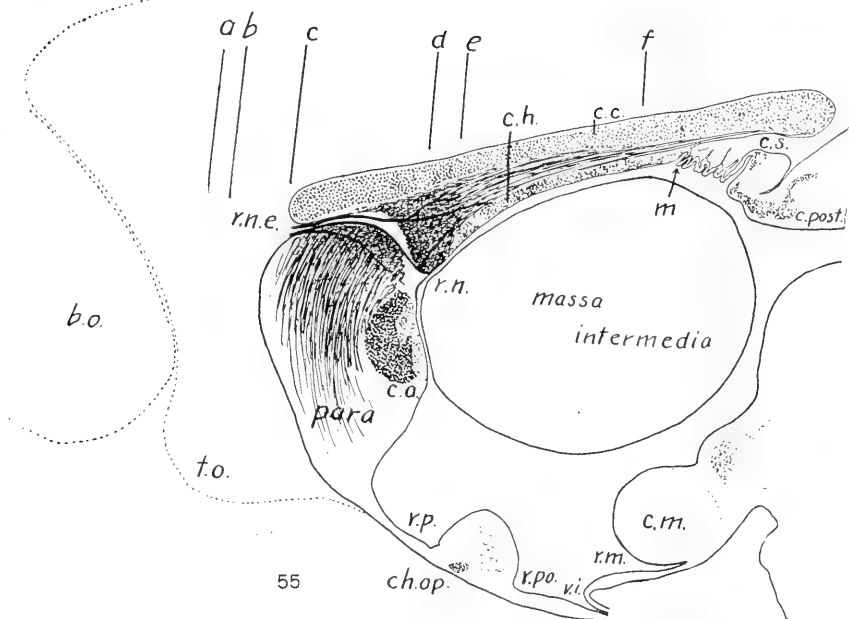
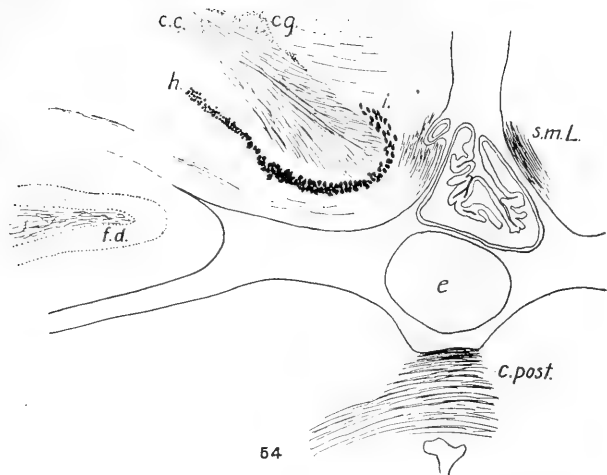


Fig. 52 Mole, transverse section through the interventricular foramen (line e in fig. 55). Description in the text.

Fig. 53 Mole, transverse section through point of junction of primordium hippocampi with the hippocampal flexure (line f in fig. 55). The right side of



the section is a little farther forward than the left, and here only the primordium is seen. The small mass beneath the commissures in the middle line is the nodulus marginalis.

Fig. 54 Mole, transverse section through the peculiar plate of large cells by which the indusium is brought into connection with the hippocampus beneath the splenium. The fascia dentata nowhere else approaches so near the splenium.

Fig. 55 Mole, median sagittal section reconstructed from several sections. Description in the text.



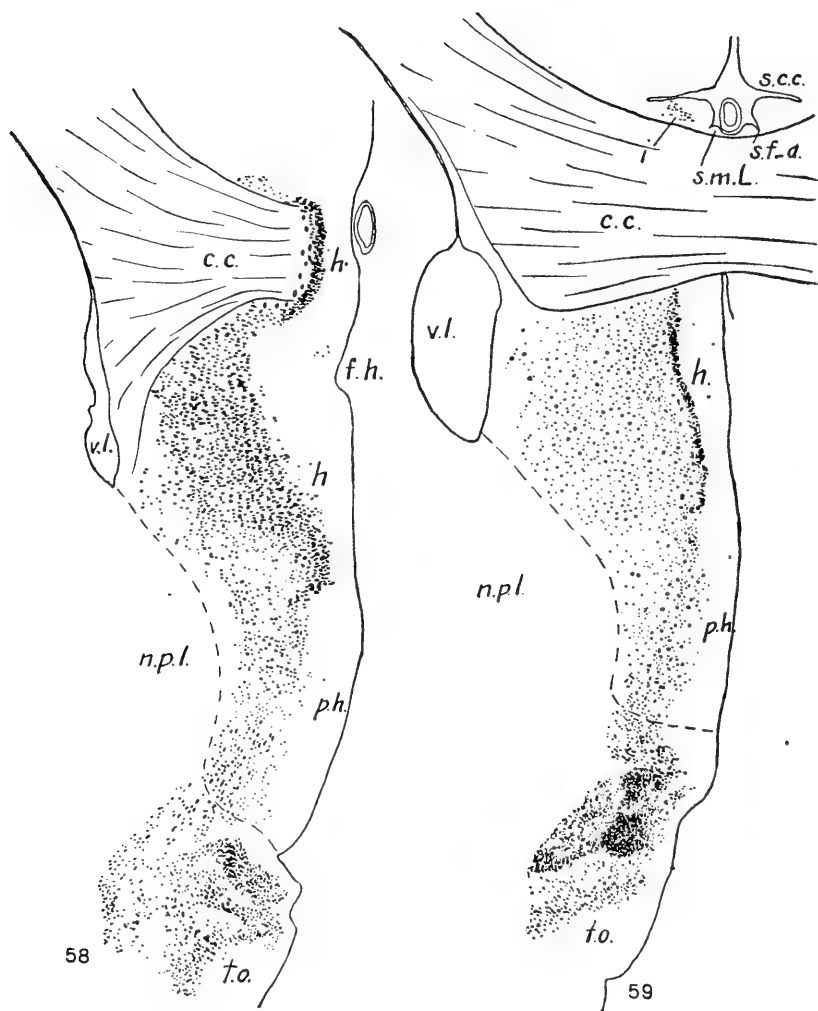
Figs. 56 to 62 Rat, seven transverse sections of the hippocampal region rostral to the interventricular foramen. Magn. 40 diam.

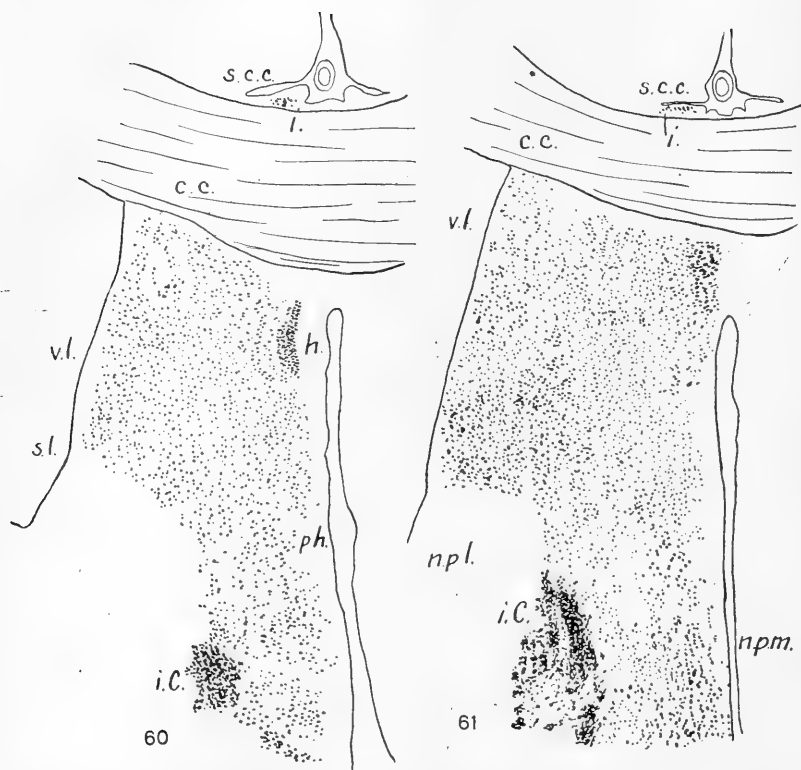
Fig. 56 Section through the rostral end of the hippocampus in the olfactory peduncle.

Fig. 57 Section through the rostral part of the olfactory tubercle showing the special medial hypertrophy of the deep cells of the tubercle. Between it and the hippocampal cortex the deep cells come out to the surface as the primordium hippocampi. In all the sections of the rat it is particularly noticeable how the primordium appears as a continuation of the deep or polymorphic layer of the hippocampus.

Fig. 58 Section showing the indusium bending around the genu. Note the large cells of the polymorphic layer. Below the genu nearly all the cells, deep as well as superficial, are pyramidal in form and there is a distinct hippocampal fissure and in-curving of the cortex.

Fig. 59 Section caudal to genu. Note the extent of the hippocampal cortex and that its deep or polymorphic layer is now part of the primordium and will continue back over the foramen as the bed of the hippocampal commissure.

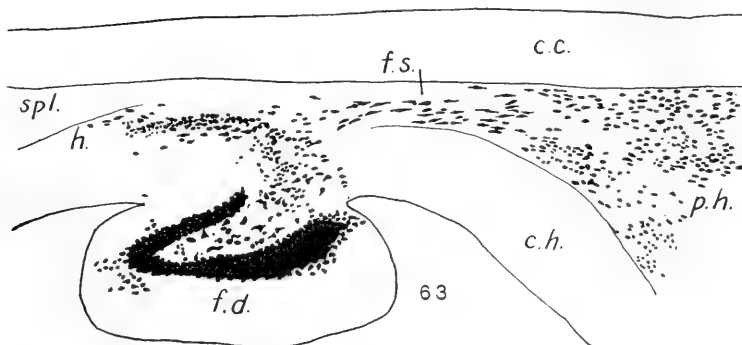
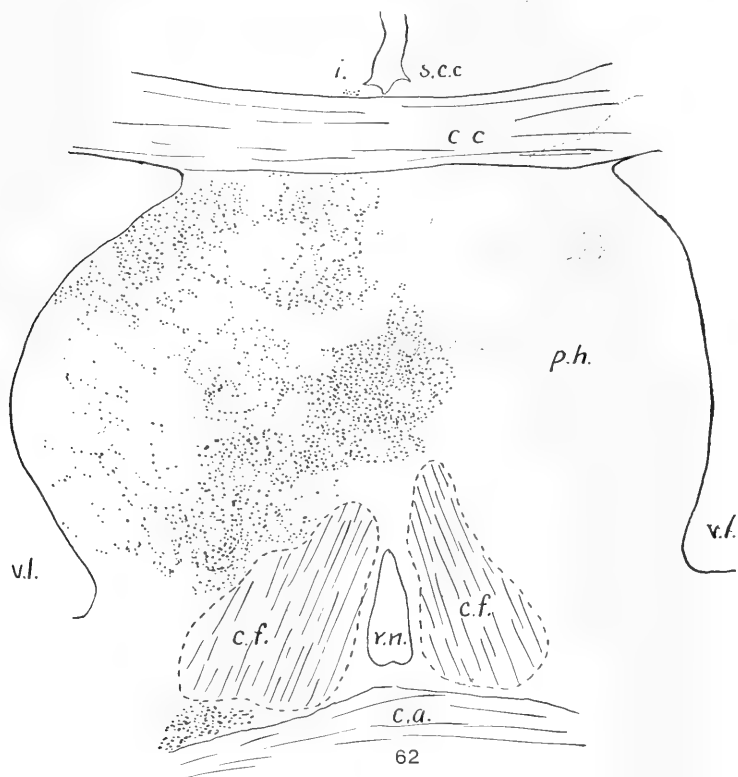




Figs. 60 and 61 Sections farther caudad. Here the hippocampal cortex below the corpus callosum becomes smaller (60) and then insensibly merges with the undifferentiated cells of the septum. In other words, we have here a hippocampus without a cortical layer and this is the equivalent of the polymorphic layer and of the primordium hippocampi of the selachian. A well developed nucleus parolfactorius medialis appears in figure 61, quite comparable to that in the bat and mole but placed somewhat more caudally.

Fig. 62 Section at the level of the neuroporic recess showing how the primordium hippocampi becomes the bed of the hippocampal commissure. On the left side where the cells are drawn, all the white space is filled by commissural fibers.

Fig. 63 Rat, parasagittal section near the median plane. The hippocampal flexure and the upper end of the fascia dentata are cut. The section cuts lengthwise of the fornix superior where that pushes between the hippocampal commissure and the corpus callosum toward the splenium. From the septum (*p.h.*) to the right many cells extend back along the fornix fibers and blend with the polymorphic layer of the hippocampus.



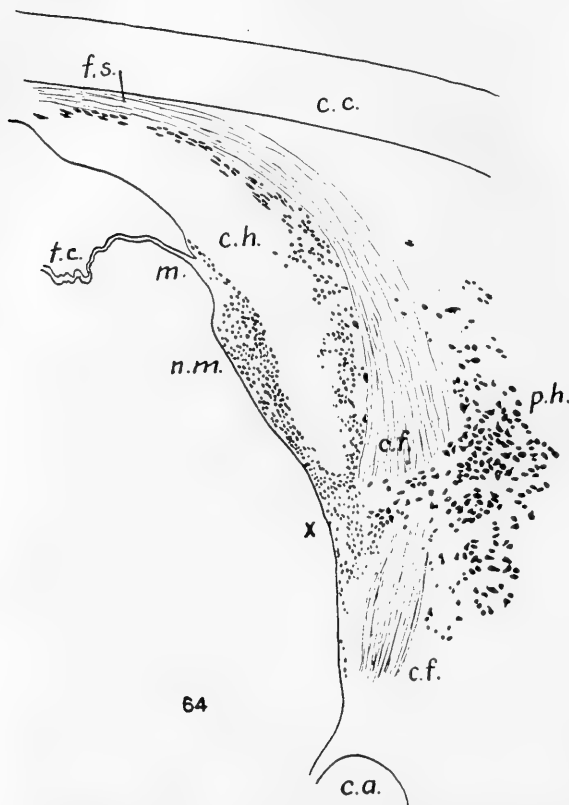
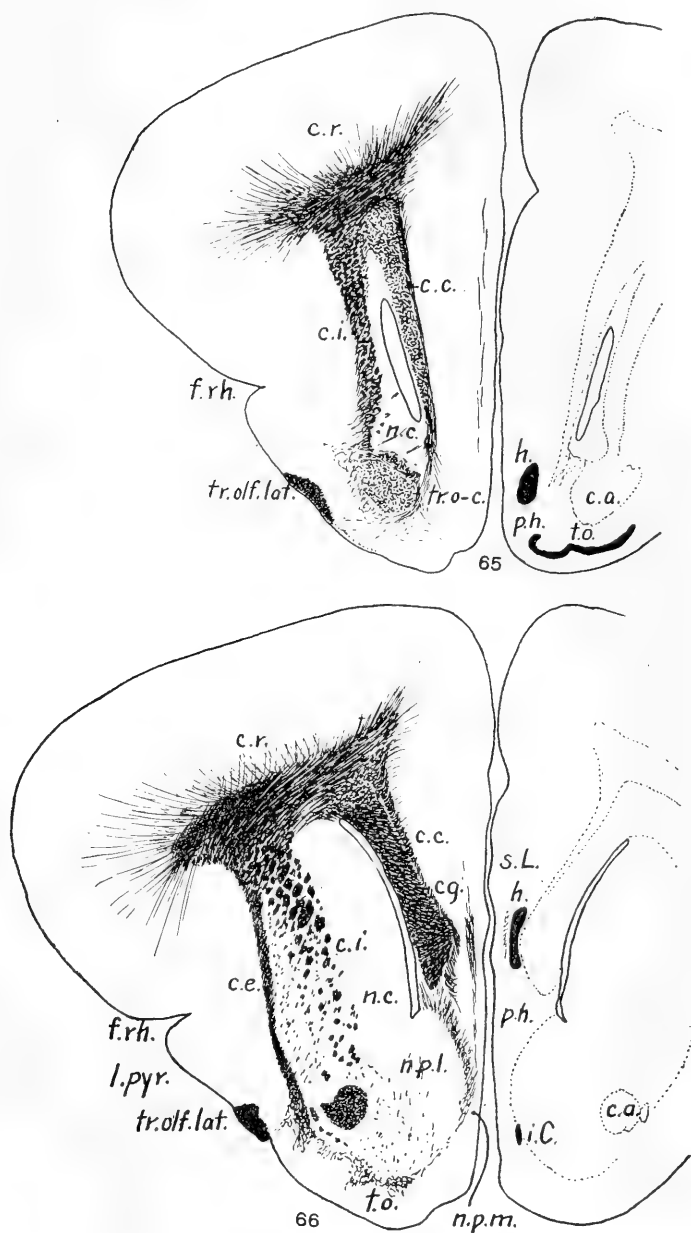


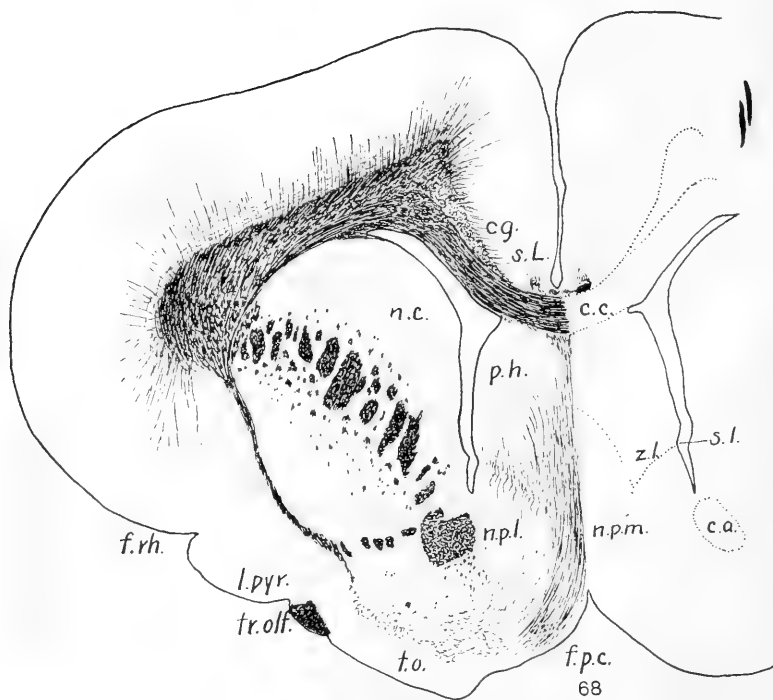
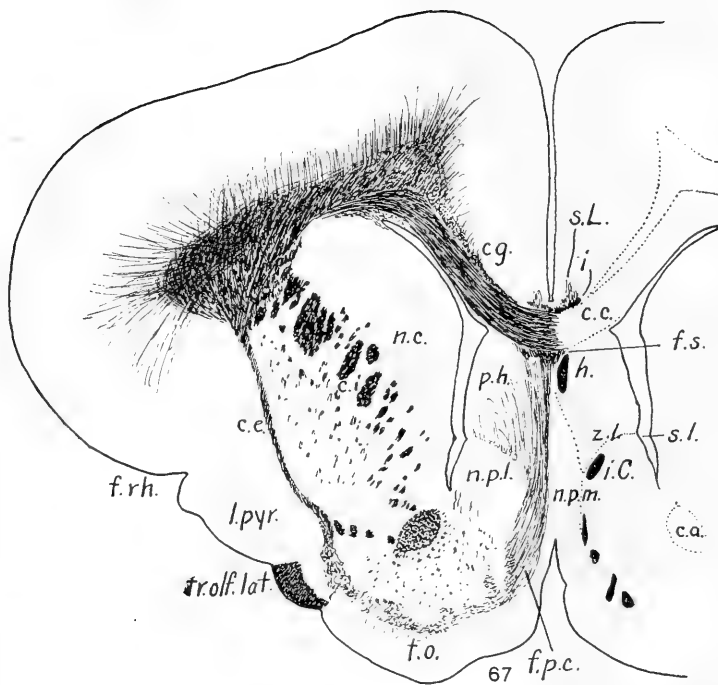
Fig. 64 Rat, sagittal section slightly inclined to the right at the dorsal edge. The section crosses the median plane at the point *x*. Above this point the section cuts the right fornix column, below it cuts the left. In front of the fornix columns (to the right) is the large-celled nucleus of the septum; behind, the small-celled nucleus in which the commissura hippocampi is imbedded. The continuity of the two nuclei between the fornix columns (as well as lateral to them) is interesting just here because there is seen at the same time a continuity with the nodulus marginalis to which the tela is attached at *m*. It is clear that this nodule is composed simply of the cells nearest the dorsal margin of the lamina supraneuroporica and that the whole commissure system has developed in the cell-mass between this and the level of the neuroporic recess.

Figs. 65 to 70 Rabbit, six transverse sections rostral to the interventricular foramen. Weigert stain.

Fig. 65 Section through the rostral end of the hippocampus near the peduncle. On the right side the hippocampus and the cortical layer of the tuberculum are shown in black. Note the olfacto-cortical fibers rising close past the caudate nucleus and the olfactory commissure bundle. Compare figure 27.

Fig. 66 Section just in front of the genu. On the right the indusium is shown (black) bending about the genu. Note the prominent band of precommissural fibers which mark the zona limitans. Compare figures 27 and 42.





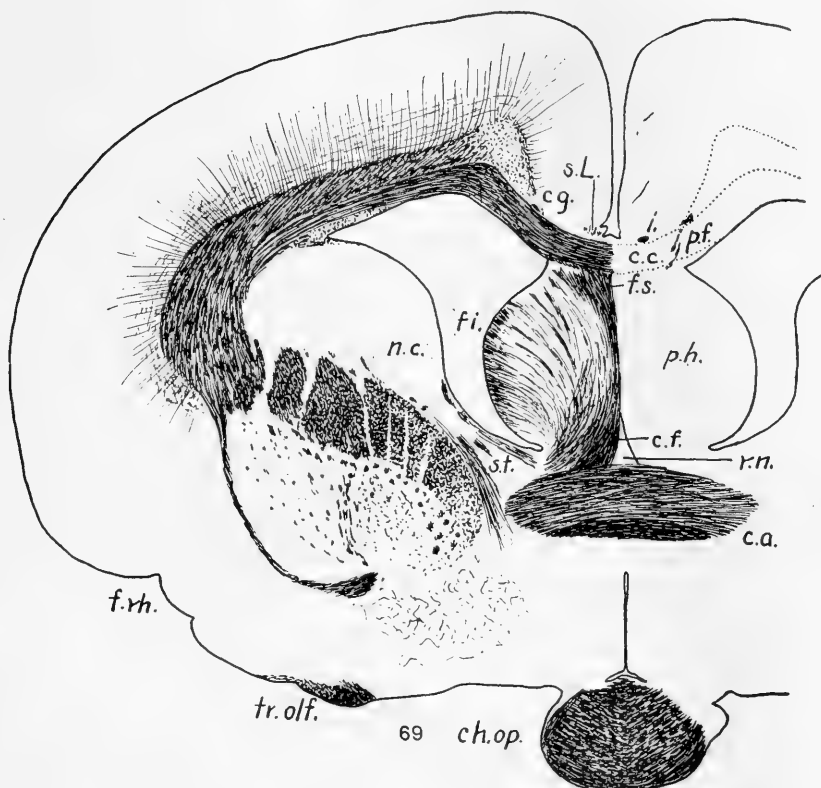


Fig. 67 Section caudal to the genu. A small band of hippocampal cortex is seen beneath the genu as in the rat. The sharp separation of the hippocampal primordium from the parolfactory area is more clear in the rabbit than in most mammals.

Fig. 68 Section a short distance in front of the anterior commissure. Note how the precommissural fibers in part contribute to the fornix superior and in part enter the hippocampal primordium.

Fig. 69 Section at the level of the neuroporic recess. The fornix columns contribute to the fornix superior. The perforating fibers appear to enter the cingulum. It is possible that they go to the hippocampus farther back.

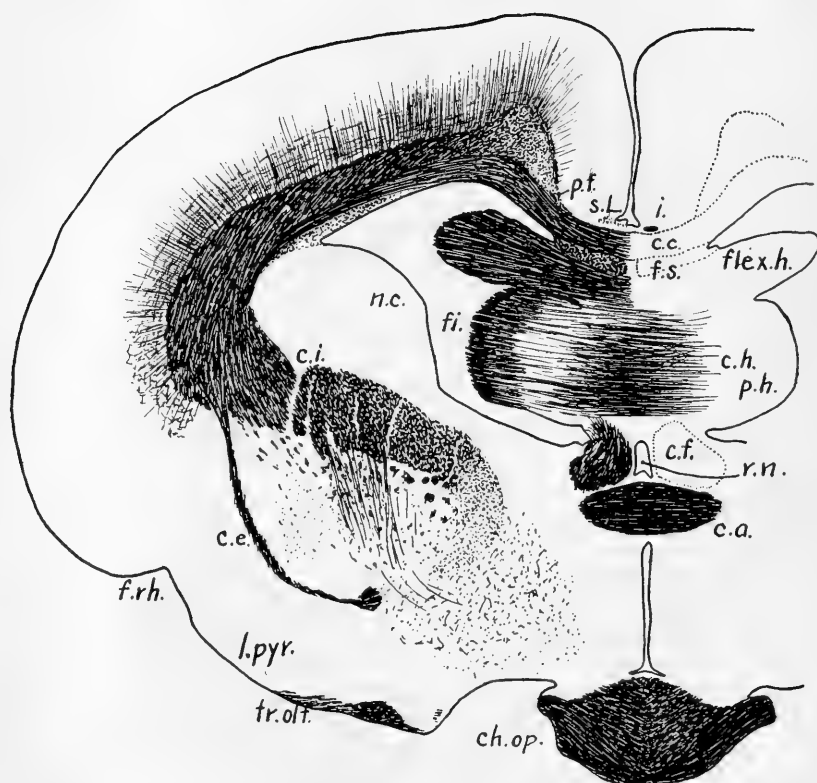
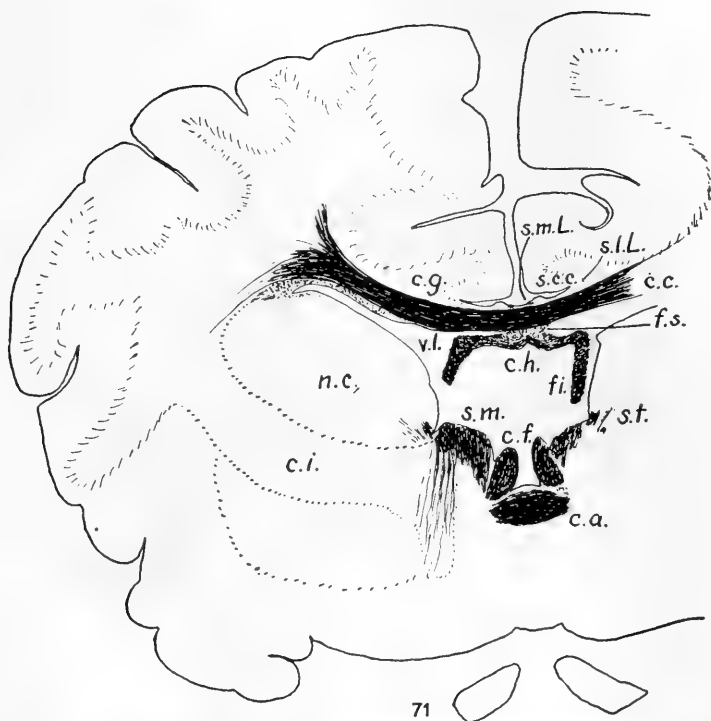


Fig. 70 Section just in front of the foramen. Description in the text.



Figs. 71 to 75 Bear (*Ursus americanus*). Five transverse sections from a Weigert series. Magn. 2 diam.

Fig. 71 Section through the interventricular foramen. The section cuts the caudal border of the anterior commissure. The base of the neuroporic recess is seen between the fornix columns. It extends forward through the whole thickness of the anterior commissure (see figs. 72 and 90). Note the prominent striae Lancisii. The fimbria is free from gray matter but a few cells accompany the fornix superior.

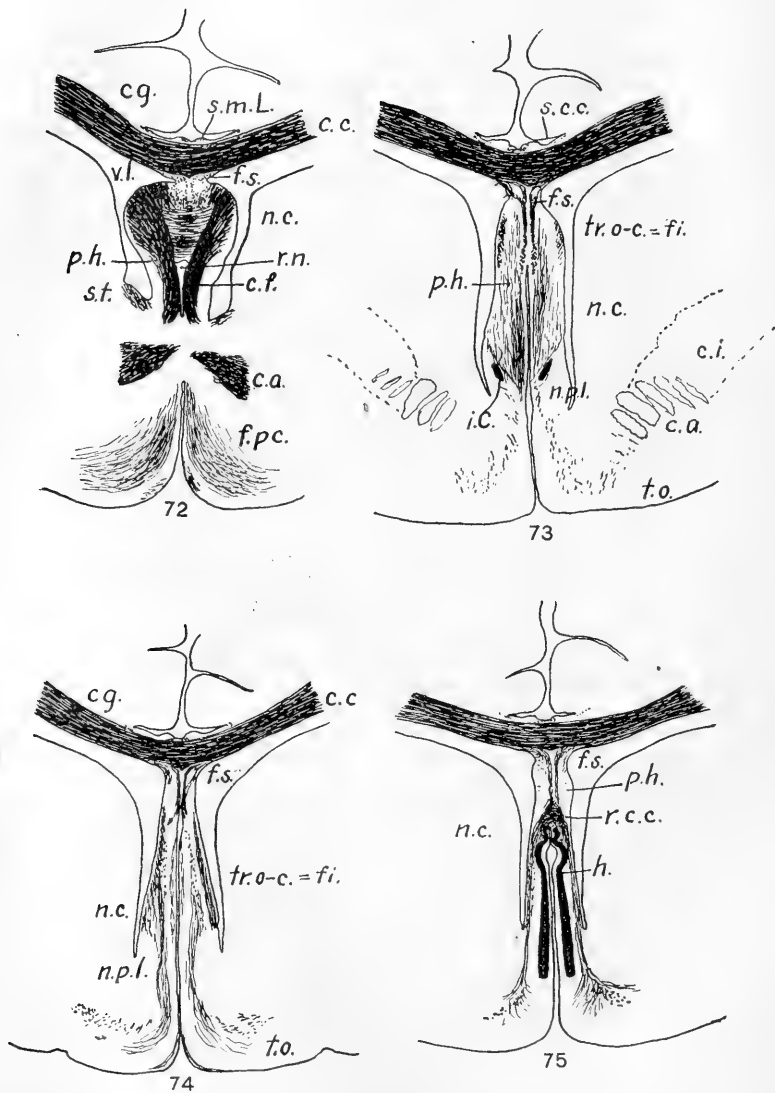
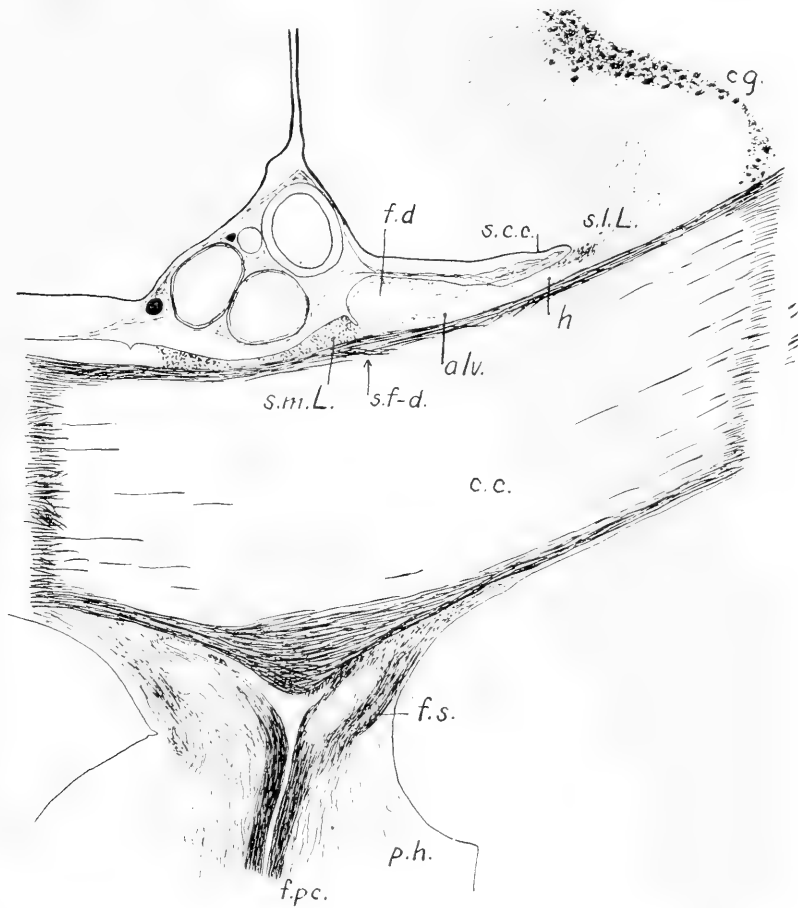


Fig. 72 Section through the hippocampal commissure, fornix columns and neuroporic recess.

Fig. 73 Section 2.15 mm. rostral to the last. The zona limitans is sharply marked by the precommissural fibers as in the opossum and rabbit. Islands of Calleja come up to this level as in the other forms.

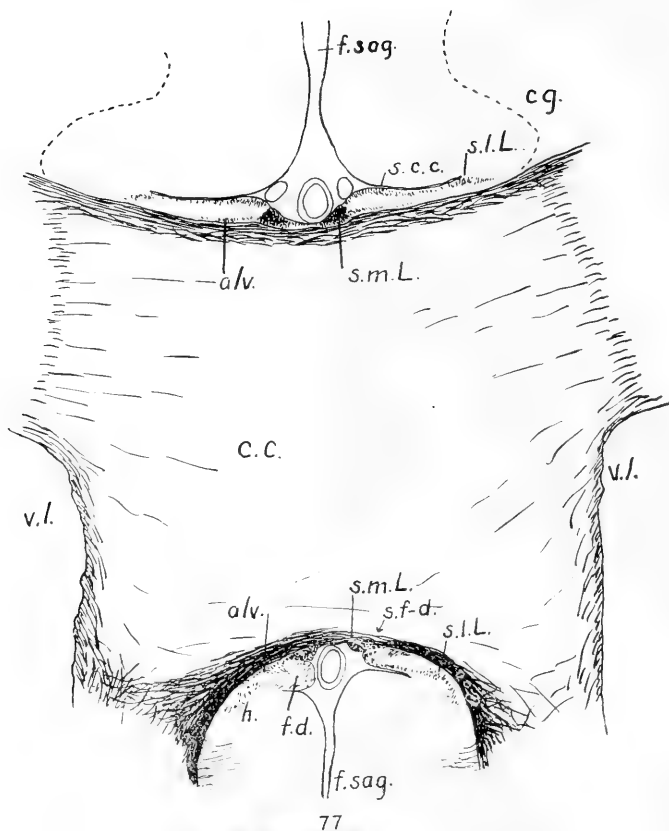


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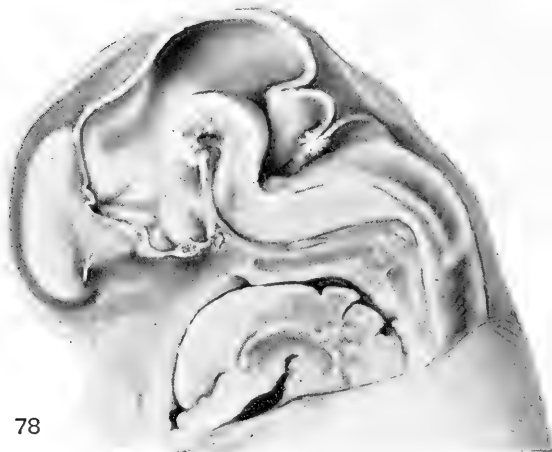
Fig. 74 Section 1.1 mm. rostral to the last. Note that in the bear the primordium hippocampi has become almost thin enough to be called a septum pellucidum.

Fig. 75 Section behind the genu and cutting the rostrum beneath. Note that here the indusium coming down around the genu as in all forms is continued down from the rostrum toward the olfactory peduncle. It is diagrammatically represented in black. This is essentially the condition of the hippocampus in the embryos of higher mammals and man.

Fig. 76 Bear, a part of the section shown in figure 73, drawn at a higher magnification. Description in the text.



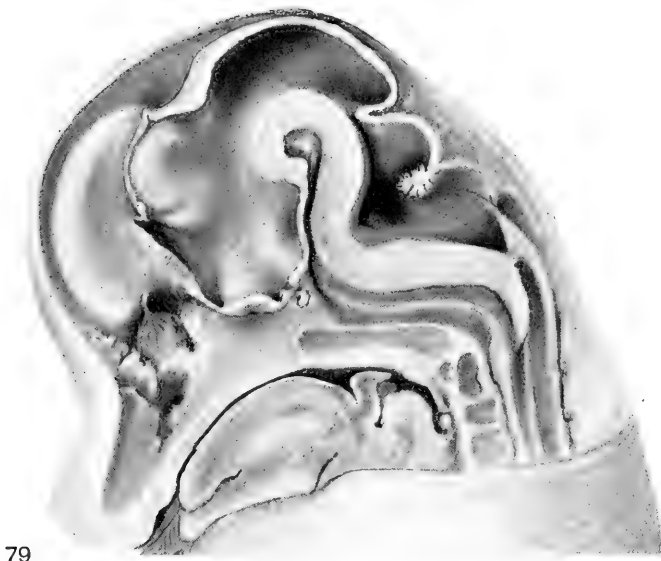
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Fig. 77 Bear, section through the genu showing that the indusium as it curves beneath the genu develops a complete, though small, hippocampal formation with all the typical parts present. Compare figures 29, 43 and 58.

Fig. 78 Pig embryo, 23 mm. Medial surface of the right half of the head. The lamina terminalis contains the anterior commissure. The lamina supra-neuroporica is only slightly thickened. Between it and the velum transversum is the paraphysal arch, the angulus terminalis of His.



79



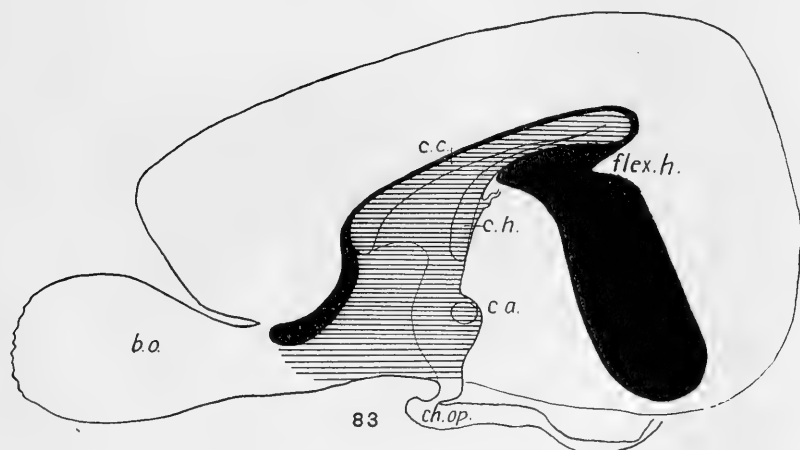
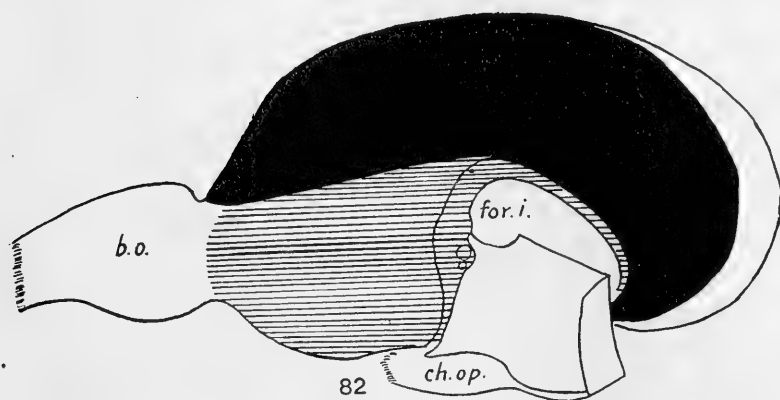
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Fig. 79 Pig embryo, 28 mm. The lamina supraneuroporica is raised to a more vertical position by the growth of the hemisphere and the thalamus is relatively crowded. There is still no commissure visible in the lamina supraneuroporica but it is somewhat thicker than in the 23 mm. stage.

Fig. 80 Pig embryo, 40 mm. The lamina supraneuroporica is still more elevated and the paraphysal arch is crowded into a deep narrow sac, in front of the developing choroid plexus. Now the anterior pallial commissure is evident as a fibrous mass occupying the lamina supraneuroporica. There is up to this time no secondary fusion nor extensive thickening in the region of the commissures.



Fig. 81 Pig embryo, 50 mm. Rapid development of the anterior pallial commissure has produced a condition very similar to that of the adult bat. The commissure still occupies the lamina supraneuroporica but also extends forward from its dorsal border to form a crescent. Above the crescent is seen the indusium in a condition very much like that of the hippocampal formation in the opossum or bat. I can find no indication of secondary fusion. The rostral limb of the commissure is formed simply by the invasion and stretching of the dorsal border of the lamina supraneuroporica by additional corpus callosum fibers.



Figs. 82 to 90 Diagrams to illustrate the relations of the hippocampus, hippocampal primordium and paraterminal body. The medial surface of the right hemisphere is drawn in each case. The paraterminal body is shaded with horizontal lines, the hippocampal primordium is distinguished by means of spindle-shaped spots or flecks and the hippocampus is in solid black. The commissures are merely outlined with pen lines. All the diagrams except those of the bat and the bear are drawn from dissections.

Fig. 82 Diagram of the brain of a reptile to illustrate the relations of hippocampus and paraterminal body as defined by Elliot Smith. The outline is taken from the turtle's brain. Herriek recognizes that the narrow ridge over the foramen in the turtle is primordium hippocampi but describes a much larger supra-foraminal portion of the paraterminal body in lizards.

Fig. 83 Diagram of the brain of the rat similar to the last. The whole commissural system is supposed to be imbedded in the paraterminal body.

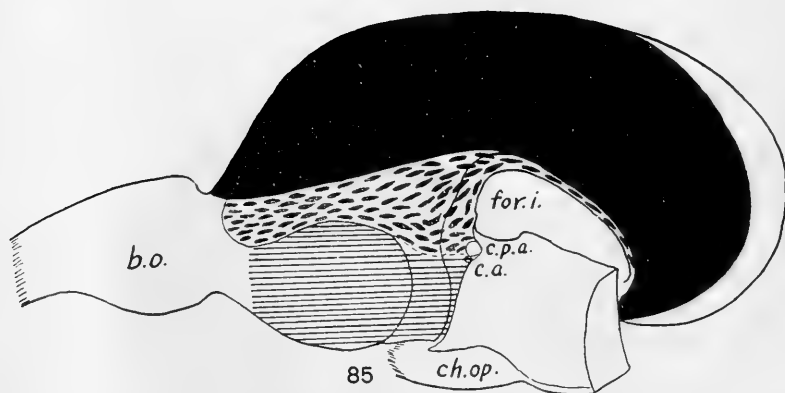
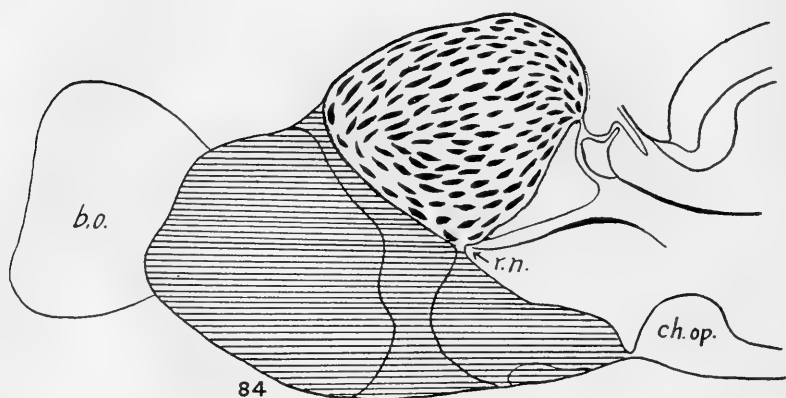


Fig. 84 Diagram of the forebrain of *Scyllium* to illustrate the author's conception of the relations. The hippocampal primordium is separated from the paraterminal body by the neuroporic recess. This primordium gives rise to two structures in higher vertebrates: hippocampal formation and a residual body less highly organized which is represented in mammals by the septum pellucidum. This residual structure is represented in the following diagrams by the flecked or spotted area.

Fig. 85 Diagram of the brain of the turtle to illustrate the view set forth in this paper. Not only in the turtle but in lizards and other reptiles the body which runs along over the foramen, between it and the hippocampal cortex, is hippocampal primordium.

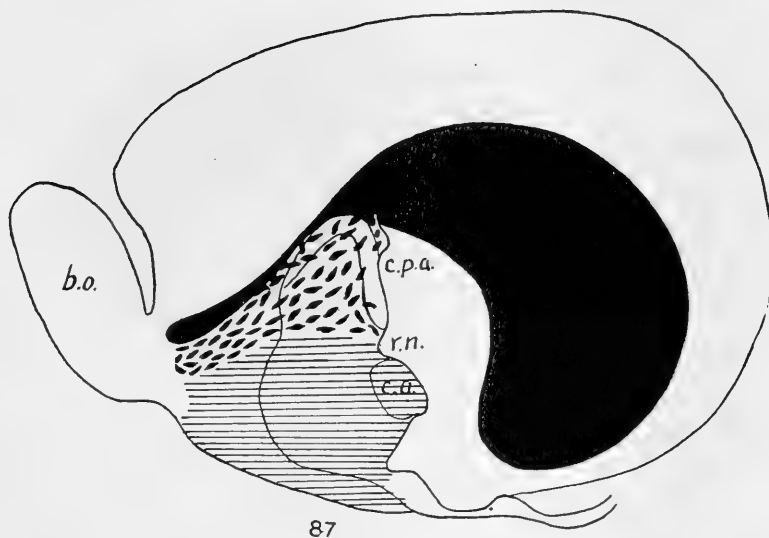
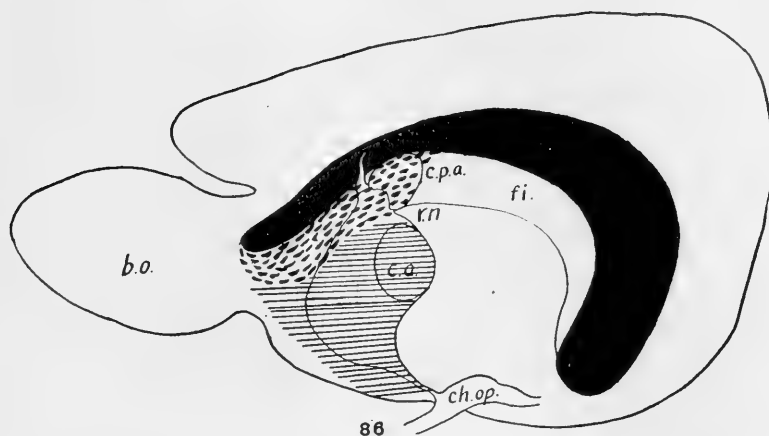


Fig. 86 Diagram of the brain of the opossum. This type of brain has been made familiar by Elliot Smith's work. The reasons for assigning a portion of Professor Smith's paraterminal body to pallial area are set forth in the text.

Fig. 87 Diagram of the brain of the bat. The commissures are poorly developed and there is nearly as close connection between hippocampus and its primordium as in the opossum.

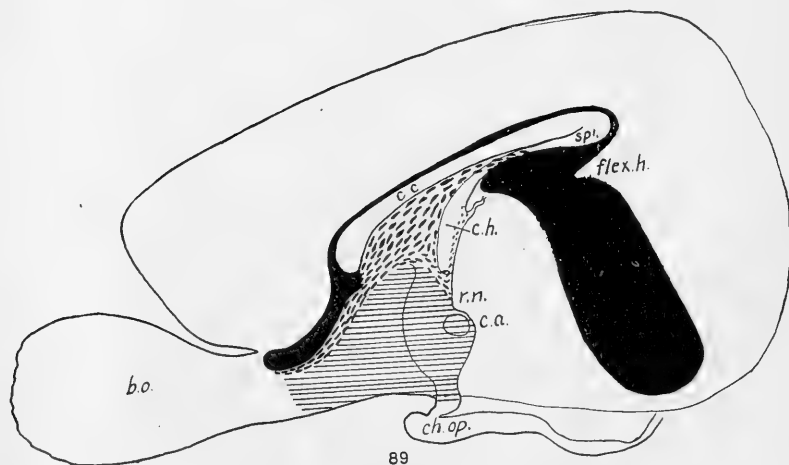
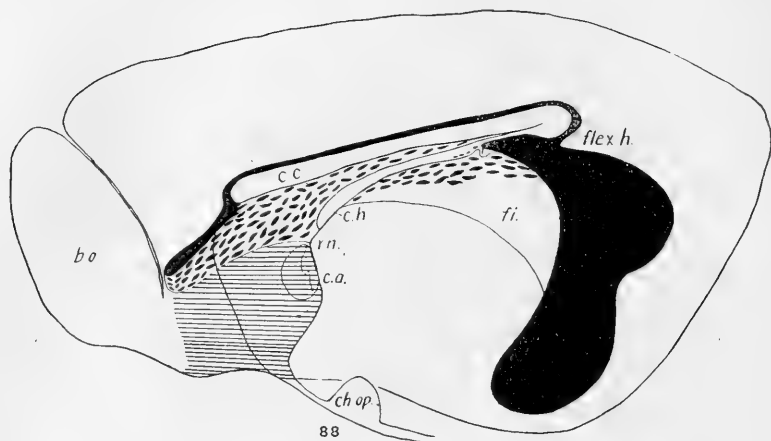


Fig. 88 Diagram of the brain of the mole. Although there is a large corpus callosum and a splenium is well formed, there is an unusually large primordium reaching almost the entire length of the corpus callosum and connecting with the hippocampal flexure. The boundary of the paraterminal body as it appears in the ventricular surface is represented here. Reference to figure 55 will show that the medial parolfactory nucleus rises much higher on the outer surface. The diagram of the rat brain following is drawn with reference to the boundary on the outer surface.

Fig. 89 Diagram of the brain of the rat. The gradual merging of the indusium beneath the genu with the primordium is represented by tooth-like projections. The paraterminal body rises higher on the medial surface than it does next the ventricle. On the ventricular surface the boundary line runs almost straight forward from the neuroporic recess.

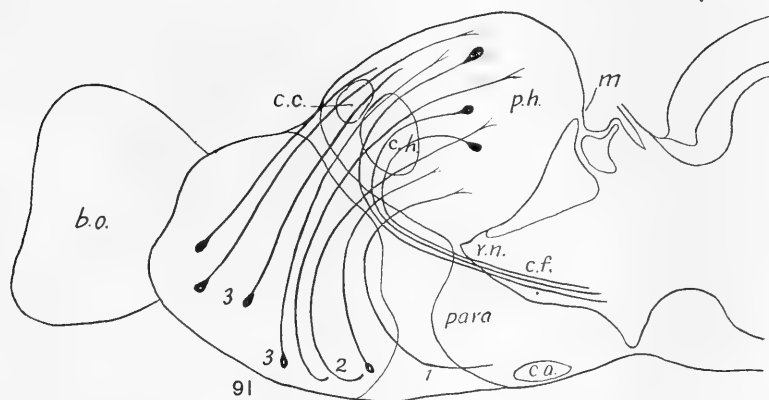
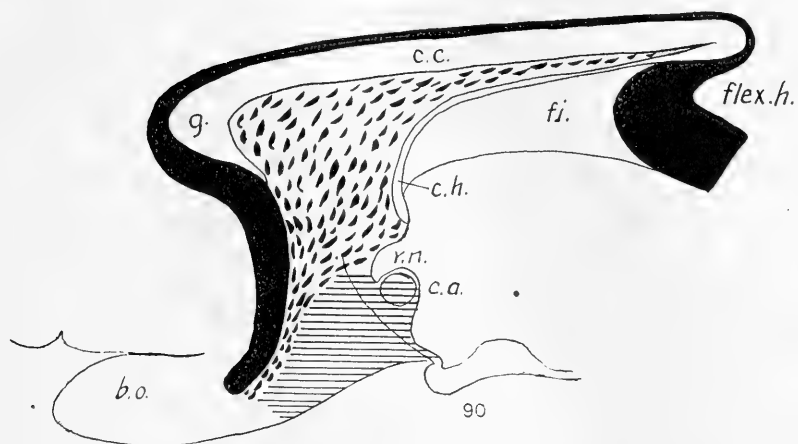
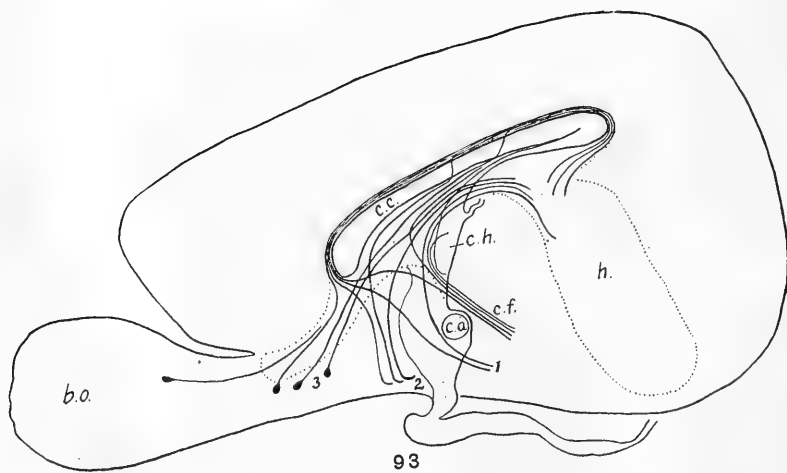
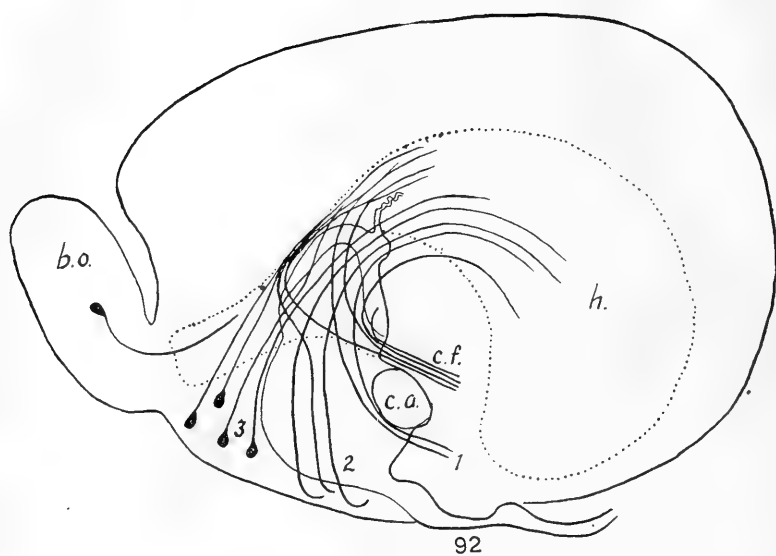


Fig. 90 Diagram of the septal region in the brain of the bear, based on serial transverse sections. The hippocampal commissure is a very thin band. The gray matter in the caudal part of the septum is reduced to a very slender strand accompanying the fibers of the fornix superior.

Figs. 91, 92, 93 Diagrams of the brains of Scyllium, the bat and the rat to illustrate the relations of the fibers of the fornix system to the pallial commissures. See section on this subject in the text. The arabic numerals 1, 2, 3, have the same significance as in figure 38.



STUDIES ON THE REGENERATION OF THE PERO- NEAL NERVE OF THE ALBINO RAT: NUMBER AND SECTIONAL AREAS OF FIBERS: AREA RELATION OF AXIS TO SHEATH

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The Wistar Institute of Anatomy and Biology

THREE FIGURES

The work here presented is part of a program of considerable magnitude, bearing upon the growth of the nervous system, now being carried out under the direction of Professor Donaldson. The experiments thus far made have already suggested so many new problems that it seems best to publish some of the results now in hand while further observations are being made.

The primary object of this study was to determine the number and size of the medullated nerve fibers within a regenerated peripheral nerve of the albino rat and compare the conditions on the side of the operation with those found in the corresponding unoperated nerve of the opposite side in the same animal.

The following series of animals furnished the data for this study:

Series 1. Animals nos. 1 to 79 inclusive.

This series was used in perfecting the technique of operation and no further record of it will appear.

Series 2. Comprised 92 animals in all. From 18 of these, sections suitable for the purposes of the study were obtained.

These 18 animals, of different known ages, were operated and after the lapse of varying periods of time were killed.

The weight at the time of operation and at the time of killing is given in all cases except one. One specimen, no. 100, was excluded because although a young animal it did not gain in weight after the operation, and two specimens, nos. 93 and 116, were excluded because they were the only representatives of their respective age groups. The

remaining specimens fell into three different age groups and presented data upon the following points:

- A. Number of fibers in left or control nerve.
- B. Number of fibers in proximal end of operated nerve.
- C. Number of fibers in distal end of operated nerve.
- D. Effect of operation upon body weight.

Series 3. Three adult unoperated animals, nos. 222, 223 and 224 of known weights were used to determine the following points:

- A. The number of fibers in the peroneal nerve of a normal animal.
- B. The difference in the number of fibers at the two ends of that portion of the peroneal nerve used in these experiments.
- C. The sectional area of peroneal nerve fibers of a normal animal and the area relation of axis to sheath.

Series 4. This series included operated animals nos. 192, 193, 210, 211 and 220. Two were adults and three were young animals of known ages; all were of known weights. They furnished data upon the progressive change in the number of fibers in operated nerves.

Series 5. This series included animals nos. 167, 168, 169, 170, 171 and 172. The ages and weights were known.

This series was used to determine when degeneration is complete.

Series 6. Animals nos. 316 and 317, Norway rats, of known weights, were used to demonstrate the equality in the number of fibers in the peroneal nerve of each side.

Series 7. Operated animals nos. 106, 114 and 154. This series was used to furnish data on the sectional area of fibers and the area relation of axis to sheath. These animals were selected from Series no. 2.

The nerve in the right leg was consistently selected for experiment and the corresponding nerve of the left leg was used as the control.

The observations made in the course of these experiments comprise the determination of the number of fibers and the area relations of axis to sheath in the left or control nerve and similar determinations at different levels above and below the point of operation on the right nerve.

The nerve selected for operation was the peroneal. This nerve is the anterior (or lateral) division of the sciatic and passes distad beneath the biceps femoris muscle in close contact with the gastrocnemius; it curves over the lateral margin of the gastrocnemius sometimes piercing the edge of this muscle to dip into the fleshy mass formed by the fused bodies of the upper extremities of the peroneal muscles (P. longus, P. brevis, P. tertius and P. quartus).

For about 10 mm. (in the adult rat) proximal to its entrance into the peroneal muscles, the peroneal nerve normally gives off no branches. This was the region selected for operation.

Distal to the region of operation, the nerve gives off branches to the peroneal muscles, the tibialis anterior, the extensor digitorum longus and also extends its fibers to the dorsum of the foot.

TECHNIQUE OF OPERATION

The method of operation was as follows: The animal was anesthetized, the nerve exposed by a cut about 1.5 cm. in length through the skin, fascia and biceps muscle. The nerve was lifted free from surrounding structures, crushed and wired or simply wired. The flaps of this wound were then closed by two or three stitches in the skin and the wound sealed by applying a bit of sterilized cotton covered with collodion.

Crushing the nerve was accomplished by means of a small pair of bone forceps, the blades of which had been polished off, leaving instead of sharp cutting edges, blunt smooth edges. By this means the bundles of white glistening nerve fibers were divided, leaving the perineurium intact as a tube connecting the divided ends of the nerve fibers. The space within the perineurium, between the divided nerve fibers, usually filled with serum or blood. This operation was followed in every case by loss of ability to extend the toes properly and to rotate the foot outward. Recovery was in most cases rapid and the repaired nerve was usually free from interfering masses of connective tissue.

The difficulties in determining at the autopsy the exact point of injury to the nerve and the importance of having this information, later led to a modification of the simple crushing technique, as follows: At the point of crushing, a silver wire hook was caught around the nerve and by means of a long narrow nosed forceps this hook was forced to close tightly on the nerve. The extended ends of the wire hook were then trimmed off, leaving a small bit of wire doubled upon itself and holding between its two limbs the perineurium or a portion of it. The

forceps especially made for this operation have long slender jaws on the inner surface of each of which at the extreme end is a transverse groove so placed that when the jaws are brought together they present opposed depressions between which the wire loop may be firmly clamped together without danger of slipping.

In later operations the preliminary crushing of the nerve by the bone forceps was omitted and the crushing done by clamping the wire hook on the nerve. No. 26 annealed silver wire was used.

The wire clamp was left on the nerve. In most cases the wire clamp remained on the nerve and became embedded in new tissue deposited about it. This new tissue formed a bridge or capsule structure connecting the proximal end of the nerve to its distal segment. This bridge was usually best developed on one side of the ring while in some cases its development formed a more or less uniform enclosure for the ring. Through this connecting bridge the fibers of the regenerated nerve were found to pass from the proximal to the distal segment of the nerve.

The animals selected varied in age from 31 days to 250 days (adults) at the time of operation and they were permitted to live from 3 to 105 days after operation.

When the animal was killed, about 10 mm. of the operated nerve, including the region of operation, was removed. An effort was made to have an equal length of nerve on either side of the clamping wire. About 10 mm. of the corresponding nerve of the left side was also removed as a control. These specimens were fixed in 1 per cent osmic acid, embedded in paraffin and sectioned. All sections were cut seven micra thick. A few sample sections of the control side were mounted. The operated (right) nerve was cut in continuous series and mounted, the series beginning in the nerve above the point of crushing where the nerve appeared to be normal, and continuing through the region of operation into the new or regenerated nerve. The sections of the operated or right nerve have been designated as proximal or distal according to their relation to the point of

crushing. Sections of the left side have been designated as left or control.

Only such cases as presented proximal, distal and control sections suitable for photographing and counting were selected.

The photographs were made with a Zeiss 8 mm. apochromatic objective and a no. 4 apochromatic eyepiece. Hardesty's ('99) method of counting fibers was employed. This consists in recording each fiber automatically by pricking a hole in each fiber image of a photographic print. The original specimen is observed under the microscope during the process. The counting was done with a Zeiss 2 mm. immersion objective, a no. 4 eyepiece and a draw tube of 160 mm. All counts and measurements were made with the same optical combination.

EFFECT OF OPERATION

In order to understand the effects of destroying the continuity of the peroneal nerve, it should be borne in mind that the fibers of this nerve are supplied to the following muscles: the peroneus longus, which passes around the external maleolus across the plantar surface of the foot and is inserted into the base of the first metatarsal—its action is to extend and slightly rotate the foot outward; the peroneus brevis, which passes around the external maleolus and is inserted into the base of the fifth metatarsal—its action is to abduct and to extend the foot; the *P. tertius* and *quadratus* fused with the *extensor longus digitorum*—their action is to extend the toes; and the *tibialis anterior* which is inserted into the base of the first metatarsal; its action is to extend the foot at the ankle and rotate the foot inward.

The immediate effect of the operation was in every case to cause a paralysis which resulted in a flexion of the toes and a rotation of the foot inward. Since no fibers of the peroneal are distributed to the *gastrocnemius* muscle and *plantar* muscle, it may be assumed that the deformity immediately following the operation is due to the action of these muscles. This deformity in many cases rapidly disappeared and in most cases after the lapse of six to ten days it was difficult to detect any abnormality in the movements of the animal.

Passing to the histological changes following the operation, we find on the fourth day after operation that all the medullated fibers distal to the lesion show appearances characteristic of degeneration. Crushing, therefore, interrupts the fibers completely. This degeneration extends from the point of crushing distally as far as observations have been made—that is, from 5 to 8 mm.—and it was assumed that the degeneration had extended to the termination of each fiber. In the other direction, degeneration extended from the point of crushing proximally from 2 to 3 mm.—according to actual measurements, from 2 to 3.2 mm. Above this point the structure of the great majority of the fibers appeared to be normal with here and there one modified in a way to suggest degenerative changes even as much as 8 mm. proximal from the point of lesion. Similar changes in structure have been figured by Boll ('76) and described as due to histological methods. I am inclined to think that the changes here observed are preparatory to the regenerative process which is about to begin and which I have found by numerical determinations to begin as much as 7 mm. proximal to the lesion. Ranson ('12) finds in dogs that non-medullated fibers degenerate more than 10 mm. up the proximal stump where regeneration begins.

Perroncito ('06) has shown that a sectioned axone begins to regenerate within three hours after cutting. In the present experiments no animals were killed earlier than three days after operation and from that period up to 105 days. No effort has been made here to trace the earliest development of the new fibers.

To determine the time when degeneration in the rat was complete, six animals were killed as follows: One 3 days after operation, two 4 days after operation and three 6 days after operation. Two of these animals were 89 days and four were 90 days of age at the time of operation. Series 5.

The three-day and the four-day animals showed complete degeneration of all fibers and the clinical signs indicated complete loss of control of muscles supplied by the peroneal. The six-day animals showed complete degeneration, but the clinical signs

were so modified as to suggest readjustment of muscular control so as to mask in a measure the paralysis produced by the operation.

The evidence from the three-day and four-day specimens seems conclusive that by the fourth day degeneration is complete.

NUMBER OF FIBERS IN PERONEAL NERVE OF NORMAL ANIMAL

Series 3 was prepared to determine, among other facts, the number of fibers in the peroneal nerve of the normal animal. Three young unoperated adults were used. Of each nerve three counts were made, one for each end and one for the middle. The distances between counts were also determined. Referring

TABLE 1

	NO. 224 ♀ UNOPERATED RIGHT NERVE, WEIGHT 104		NO. 223 ♀ UNOPERATED RIGHT NERVE, WEIGHT 117		NO. 222 ♂ UNOPERATED LEFT NERVE, WEIGHT 182	
First or proximal count.....	2240		2430		2192	
Distance in μ from 1st to 2d count.....		3031		4746		3080
Second or middle count.....	2118		2292		2418	
Distance in μ from 2d to 3d count.....		4466		2345		3332
Third or distal count.....	2392		2213		2364	
Average count.....	2250		2312		2325	
Combined average count, 2296			Average of the distal counts, 2323			
Average of the proximal counts, 2288			Average body weight, 135			
Average of the middle counts, 2276						

to table 1, we note that the average number of fibers at the three levels in no. 224 is 2250, in no. 223 it is 2312 and in no. 222 it is 2325. The combined average counts of all three specimens is 2296 for an average body weight of 135 grams.

Various studies on the peripheral nerves by others (Dunn '00) indicate that substantial symmetry exists normally between the nerves of the two sides of the body in respect to their numerical composition. This view is supported by our own direct observations.

TABLE 2

UNOPERATED NORWAY RAT NO.	NUMBER OF FIBERS	
	Right nerve	Left nerve
316 ♂	1990	2031
317 ♀	2055	2002
	1930 ¹	
Average.....	1992	2017

¹ Second and more distal count.

Table 2 presents the results of the determinations of the number of fibers in the right and left peroneal nerves of two specimens of the Norway rat (*Mus norvegicus*) from which the albino strain has been derived.

Two counts were made, at different levels, in the case of the right peroneal of no. 317; one count was made for each of the other nerves. The average number of fibers in the right peroneal nerves is 1992, in the left 2017—a difference of 25 fibers or about 1.5 per cent. Since this difference is within the probable limits of normal variation (2 per cent) we may conclude that the right and left peroneal nerves contain approximately the same number of fibers.

These data on the Norway rat have been introduced here merely to show the similarity of the number of medullated fibers in the peroneal nerve on the two sides of the same animal. The difference in the absolute number of medullated fibers in this nerve in the Norway rat and in the albino calls for a special study.

Returning to table 1, we note that the average of the proximal counts of the three nerves is 2288 and that the average of the distal counts of the three nerves is 2323, showing an average excess of 35 fibers in the distal end of the nerve or an increase of about 1.5 per cent in 10 mm. of nerve. Taking the average of the two extremes 2288 and 2323 we get 2306 as the estimated number in the middle portion of the nerve. The average of the observed numbers of the middle counts is 2276, a difference of a little more than 1 per cent. We can, therefore, safely assume that 2306, the estimated number of the middle count is approxi-

mately the normal number of fibers for the middle zone of the peroneal nerve of the albino rat with a body weight of 135 grams and belonging to the strain here used.

Referring again to table 1, we observe that no. 224 weighed 104 grams, no. 223 weighed 117 grams and no. 222 weighed 182 grams or 75 per cent more than no. 224. The average of the three fiber counts of no. 224 is 2250, the average of no. 223 is 2312 and the average of no. 222 is 2325 or an increase of about 3.5 per cent over the average of no. 224. In this series, therefore, the heavier animal has the greater number of medullated fibers.

Taking body weight as a rough index of age, we note that there is a constant increase in the number of fibers as the animal grows older. With an increase of 75 per cent in body weight we find an increase of about 3.5 per cent in the number of fibers in the peroneal nerve. This observation must be held to apply to young or developing animals for Dunn ('11) has shown that old animals lose fibers.

Summarizing the observations on the normal peroneal nerve we may state that: In an animal of 135 grams body weight the middle zone of the peroneal nerve contains about 2306 fibers; the number is the same for each side; in a developing animal an increase in the number of fibers accompanies increase in body weight or advancing age; in the 10 mm. of nerve used in this series there is an increase of about 1.5 per cent in the number of fibers as we pass from the proximal to the distal end. Dunn ('02) has shown that in the frog there is an increase in the number of fibers between the sciatic trunk and its two distal divisions of about 5 per cent, due to branching.

NUMBER OF FIBERS ON THE CONTROL SIDE OF OPERATED ANIMALS

Having examined the numerical relations in the peroneal nerve of the normal animal we will next examine the control (left) side of our operated animals.

Table 3 presents the data from Series 2, all of which are operated animals. In this table the data are arranged according to the age of the animal at the time of killing.

TABLE 3

SERIAL NO.	AGE AT TIME OF		TIME ELAPSED	WEIGHT AT TIME OF		GAIN IN WEIGHT	NUMBER OF FIBERS			DISTANCE IN MICRA FROM LESION TO SECTIONS COUNTED	
	Operation	Killing		Operation	Killing		Cont. left	Prox. right	Dist. right	Proximal	Distal
1 M. 93 ¹	38	65	27	62.5	133.0	70.5	2200	3035	3188	2877	1274 R ²
2 M. 146	31	128	97	43.0	184.3	141.3	1995	2085	3305	7399	3122 F
3 M. 151	31	128	97	49.0	148.0	99.0	1995	4176	3792	2436 ³	1281 ³ R
4 M. 154	31	129	98	31.5	162.5	131.0	1943	2887	2662	5754	2044 ³ R
5 M. 150	31	129	98	32.5			1984	3426	2594	4236	1582 ³ R
6 M. 147	31	129	98	45.5	170.0	124.5	1987	3366	3453	4363	1645 ³ R
7 M. 142	67	156	89	127.0	183.0	56.0	2068	2400	4711	4893	2198 ³ R
8 M. 110	56	159	103	85.5	183.5	98.0	1995	4609	3202	735	1687 R
9 M. 111	56	159	103	80.0	129.5	49.5	2100	2063	3325	6099	1722 ³ R
10 F. 118	58	161	103	119.5	153.0	33.5	2070	3188	2051	406 ³	14 ³ R
11 F. 113	59	164	105	103.5	140.0	36.5	1937	2168	4262	910	896 R
12 F. 114	59	164	105	111.5	157.0	45.5	1981	2932	3177	5277	990 ³ R
13 F. 112	59	164	105	101.5	160.0	58.5	2020	2188	4188	5868	2611 ³ F
14 M. 100 ¹	61	166	105	73.5	75.0	1.5	1938	1999	18	2632	3493 R
15 M. 116 ¹	90	193	103	128.0	152.5	24.5	2138	4351	3680	1953	1946 R
16 M. 105	250	276	26	161.5	153.5	-7.0	2076	2252	361		I
17 M. 106	250	276	26	175.0	148.0	-27.0	2018	3412	3341		R
18 M. 107	250	276	26	151.0	144.0	-7.0	2155	2274	19		I
Average.....					158.3	2022	2895	2963		

¹ Record omitted in making averages of number of fibers.² R, animal recovered; F, fair recovery; I, imperfect recovery.³ Not all sections were counted.

If we divide these records into age groups, as is done in table 3, we find that five such groups are formed, namely: a 65-day group, a 128-129-day group, a 156-166-day group, a 193-day group and a 276-day group. For the 65-day and the 193-day groups only one entry is available, these, therefore, may be excluded. Owing to the failure of the 166-day animal (no. 100) to gain in weight, this will be omitted in the discussion, since it may be safely assumed that whatever has interfered with the growth of the animal has also modified the regeneration process.

This leaves for comparison the 128-129-day group with 5 entries, the 156-164-day group with 7 entries and the 276-day group with 3 entries. Bringing the averages of these groups together in tabular form we have the following:

TABLE 4

	AVERAGE AGE	AVERAGE WEIGHT		AVERAGE NUMBER OF FIBERS IN		
		Before operation	When killed	Control nerve	Operated Nerve	
					Proximal	Distal
128-129-day group.....	129	40.3	166.2	1981	3188	3162
156-164-day group.....	161	104.1	158.0	2025	2793	3560
276-day-group.....	276	162.5	148.5	2083	2646	1241

The average number of fibers in the control nerves of each of these groups is respectively 1981, 2025 and 2083. This indicates a steady increase in the number of medullated fibers with advancing age, the interval being 147 days between 129 and 276 days of age. The difference in number of fibers between the extremes is 102 or about 5 per cent of the smallest number.

Comparing the averages of these three groups with the records of normal unoperated animals, as presented in table 1, we observe that the averages of operated animals fall below the counts of even our lightest unoperated animal no. 224.*

Taking 2306 as our estimated number of fibers for the middle zone of an unoperated animal of 135 grams body weight and comparing this number with the number of fibers (1937) found in the control nerve of operated animal no. 113 of 140 grams body

weight (table 3) we note that our operated animal has 369 less fibers than the normal animal, a difference of 16 per cent, the normal number being taken as the standard.

It is, therefore, evident that the operation causes in this instance a loss of about 16 per cent of the medullated fibers in the control nerve.

If further we compare the grand average of the number of fibers in the control nerve 2022 (table 3) with the estimated normal number 2306, we still find a deficiency of 284 fibers or 12.3 per cent.

It thus appears that there is a substantial deficiency in the number of the medullated nerve fibers in the control nerve of the operated animals.

Again, if we note the average weights of the different groups at the time of operation and at the time of killing as shown in table 4, we see that the 276-day or oldest animals lost weight, while the two younger groups gained weight between the time of operation and the time of killing. We also observe that at the time of operation the younger the group the less is its average weight, while at the time of killing the younger the group the greater is its average weight, showing that the effects of operation were more profound on the older animals.

Dunn ('09) found a less number of efferent fibers in the unoperated leg of an operated frog, but apparently did not attribute the loss to the treatment the frog had received. So far as I am aware, there are no records in the literature touching the loss of fibers in the intact nerve as a result of an operation. Appreciating the importance of these effects of operation upon the unoperated nerves, experiments have been extended along this line and the results will be presented in a later study.

NUMBER OF FIBERS IN THE OPERATED NERVE

We will now pass to the consideration of the relations to be found in the operated peroneal nerve. The data to be discussed are presented in table 3, which gives the number of fibers found on the proximal and the distal sides of the lesion; the position

of the counts as measured from the point of lesion; the time elapsed and the gain or loss in weight since the operation.

We see from this table that the number of fibers found on the proximal side of the lesion in the regenerated nerve 27 to 105 days after operation is always equal to or greater than that of the corresponding control, since the one instance (no. 111) in which this number of proximal fibers is recorded as less than that in the control is quite within the limits of normal variation, about 2 per cent. On the distal side, however, there are several instances in which the number of fibers is distinctly less than in the corresponding control nerve, although in most cases it is largely in excess.

Considering first the nerve proximal to the lesion, we find in table 3 twelve instances (omitting no. 100) in which the distance

TABLE 5

DISTANCE PROXIMAL FROM LESION	NUMBER OF CASES	AVERAGE NUMBER OF FIBERS
From 8 to 6 mm.....	2	2074 ¹
From 6 to 4 mm.....	6	2866 ²
From 4 to 2 mm.....	1	3035 ³
From 2 to 0 mm.....	3	3709 ⁴

¹ The control average at this level is 2047

³ The control average at this level is 1938

² The control average at this level is 1997

⁴ The control average at this level is 2023

from the lesion to the level of the section has been measured. A preliminary study of these enumerations arranged in the order of their distance from the lesion showed that there was an evident though irregular increase in the number of fibers as the section approached the level of the lesion.

When we take the average of these records in successive groups as they appear at intervals of 2 mm., we obtain the values given in table 5.

This table shows an increase of 1635 in the number of fibers between the extremes or approximately 79 per cent of the smaller number or 80 per cent of the most proximal control average as we pass from a mean point 7 mm. proximal to the lesion toward the immediate neighborhood of the lesion.

Passing next to the distal side of the lesion and dividing the five available records into two groups, one group including the enumerations between the lesion and 1.5 mm. distal to the lesion, the other group including those between 1.5 mm. and 3.1 mm. distal to the lesion and omitting all those in which sections were lost, as well as the records of no. 100, we obtain the following:

TABLE 6

DISTANCE DISTAL TO THE LESION	NUMBER OF CASES	AVERAGE NUMBER OF FIBERS
From 0 to 1.5 mm.....	2	3725 ¹
From 1.5 to 3.1 mm.....	3	3396

¹ The control averages for these groups are 2069 and 2067 respectively.

Table 6 shows that there is an 80 per cent increase in the number of fibers over the control average in the first group located between 0 and 1.5 mm. distal to the lesion and a 64 per cent increase over the control average in the second group located between 1.5 and 3.1 mm. distal from the lesion.

From these enumerations it is evident that, following the complete degeneration which extends from 2 to 3.2 mm. proximally from the lesion there occurs in the course of regeneration a branching growth of the axis cylinders which appears to take place at considerable distance above the point at which complete degeneration terminates. This branching results in an increase of about 80 per cent in the number of medullated fibers as the region of the lesion is approached; this increase diminishes as the distance distal to the lesion increases. This decrease in excess fibers distal to the lesion is probably due to the failure of a portion of the fibers to continue their development for any considerable distance beyond the lesion.

In specimen no. 100, age 61 days at the time of operation, it will be noted (see table 3) that when killed, 105 days after operation, the animal had increased in weight only 1.5 grams. This failure to grow is also accompanied by a reduction in the number of regenerated fibers most marked on the distal side of the lesion where at a distance of 3.4 mm. from the lesion only 18 medul-

lated fibers could be found. At 2.6 mm. proximal to the lesion 1999 fibers were found the control for this nerve being 1938. Similar conditions exist for nos. 105 and 107. In these cases, however, the animals being adults, the results are not so indicative of profound disturbance in the growth processes.

The data given in tables 5 and 6 have been used for the construction of figure 1. In this figure the intent is to show by the length of the transverse lines the number of fibers at the given sectional level of the nerve; the location of the count proximal or distal to the lesion is shown by the position of the transverse line and is measured in micra by the scale on the left, from zero, the point of the lesion. The solid portions of the transverse lines indicate the number of fibers in the corresponding left or control nerves—for the most part taken from the middle zone of the control nerves—while the broken line prolongations complete the representation for the operated nerve. The values of these lines are given by the scale, "number of fibers" at bottom of the figure. In entering the data from tables 5 and 6, the distance from the lesion used in the figure is intermediate between the limiting values as given in the tables.

CONFIRMING COMPOSITE RESULTS BY A NUMBER OF DETERMINATIONS ON THE SAME INDIVIDUAL

Since the foregoing results are composite or based upon single determinations made on the proximal and distal segments of the operated nerves of a series of different animals, it seemed advisable to verify them by a number of determinations made at different levels on the operated nerve of the same animal.

For this purpose Series 4 was operated and prepared. The data relating to this series are presented in table 7 and arranged according to the weights of the animals. The table gives the counts made at different levels and indicates in micra the distances between the successive counts and also between the lesion and the nearest proximal and the nearest distal counts.

Referring to table 7, we observe that in each instance there is a large increase in the number of fibers as we pass from the first to the third count; the gain in number amounting to 249

per cent of the first count in the case of no. 211, while no. 193 shows the lowest increase of 106 per cent.

The largest number of medullated fibers is found in each case just proximal to the lesion. Distal to the lesion the number of fibers found, while greatly in excess of the normal, is somewhat less than the number found just proximal to the lesion, and still less in the most distal determinations. Thus it is seen that some of the regenerated fibers fail to find their way through the scar tissue in the region of the lesion.

TABLE 7

	NO. 193 OPERATED AGE 127, WEIGHT 64		NO. 220 OPERATED AGE 121, WEIGHT 84		NO. 192 OPERATED AGE 127, WEIGHT 117		NO. 210 OPERATED ADULT, WEIGHT 184		NO. 211 OPERATED ADULT, WEIGHT 198	
First or proximal count...	2560		2091		2280		2400		2144	
Distance from first to second count, in μ ...		2982		3045		3675		3969		3661
Second count...	2788		2426		2570		2632		2472	
Distance from second to third count, in μ		3115		2485		3278		3164		3430
Third count...	5260		5725		4158		7611		7497	
Distance from third count to lesion, in μ		448		938		637		553		595
Point of lesion										
Distance from lesion to fourth count, in μ				2478				1232		1225
Fourth count...			3991				5150		4870	
Distance from fourth to fifth count, in μ				1442				938		1981
Fifth count...			4272				4390		3140	

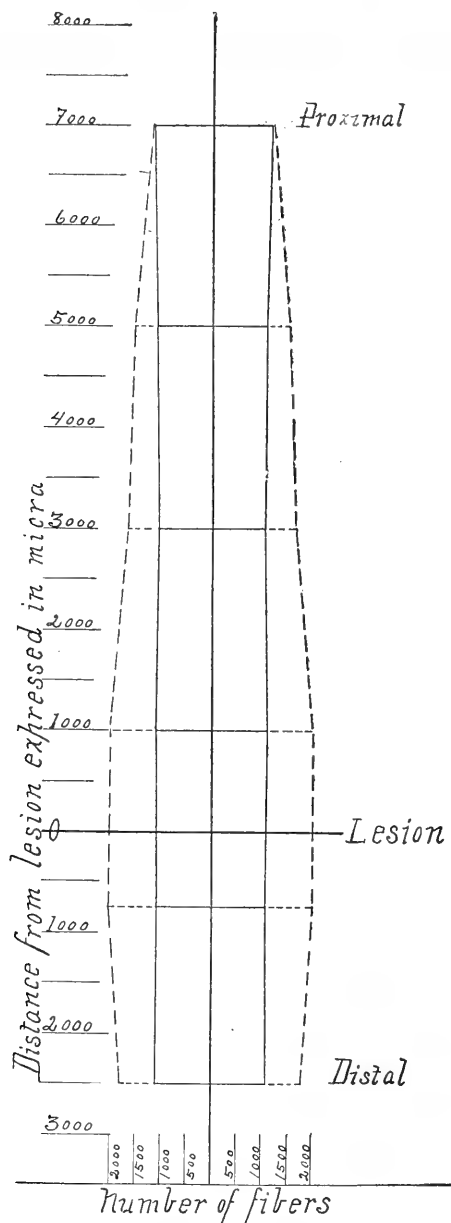


Figure 1

To put the results into more condensed form, we have arranged table 8.

TABLE 8

NO.	WEIGHT	DAYS ELAPSED SINCE OPERATION	FIRST COUNT	THIRD COUNT	DISTANCE FROM FIRST COUNT TO LESION	GAIN IN FIBERS
	<i>grams</i>				<i>micra</i>	
193	64	79	2560	5260	6545	2700
220	84	76	2091	5725	6468	3634
192	117	79	2280	4158	7590	1878
210	184	77	2400	7611	7686	5211
211	198	77	2144	7497	7686	5323
Average...	129	77	2295	6050	7195	3749

If we deal with the averages of this table, we note that the first counts were 7195 micra proximal to the lesion or practically 7 mm. and that the average of the first counts is 2295. Referring to table 3, which presents the data of an operated series and is, therefore, comparable with the data of table 8, we note that the average number of fibers in the control of this series is 2022. Thus we see that the average number of fibers in the operated nerves 7 mm. above the lesions is more than 13 per cent greater than the average of the controls given in table 3.

It should be borne in mind that the number of fibers increases with the body weight in growing animals. The average body weight in the series of table 8 is 129 grams, while the average weight of the series of table 3 is 158.3 grams. But, nevertheless, the series of table 8 has the greater average number of fibers.

We conclude, therefore, that more than 13 per cent of the increase in number of fibers of the regenerated nerves takes place at a point higher than 7 mm. proximal to the lesion.

Referring to table 7, it will be observed in no. 220 that the fifth count (distal to the lesion), unlike that of no. 210 and 211, exceeds the fourth by 281, so that instead of a decrease in number as we pass distally from the lesion there is in this case a slight increase. This is probably due to the fact that medullation has begun to disappear from some of the excess of new

fibers, and that this disappearance of myeline has taken place from above downward. This point will be dealt with in another study.

Figure 2 presents in graphic form the increase in the number of fibers of the regenerated nerves of Series no. 4, the data for which is to be found in table 7. Nos. 192 and 193 are, however, not recorded in the figure since in each case no counts were made beyond the lesion.

As in figure 1, the length of each transverse line indicates the number of fibers and its position, measured by the scale on the left from 0, the point of operation, tells the level at which the count was made. There are no data for the control nerves.

The solid line, the broken line and the dotted line are used to connect the entries for nos. 220, 210 and 211 respectively.

Figures 1 and 2 and the tables on which they are based show similar numerical relations within the regenerating nerve so that we may conclude that there is a progressive increase in the number of fibers from a point at least 7 mm. above the point of operation to the level of the lesion, followed by something of a loss in the next few millimeters distal to the lesion.

SECTIONAL AREAS: AREA RELATION OF AXIS TO SHEATH

Numerous methods have been used for determining the diameter, sectional areas and number of fibers in nerves. These methods were summarized by Vashkevitch ('89), since which time considerable improvement has been made in optical devices for this work.

The method which appeals to me as the most accurate is to measure a drawing of the projected and highly magnified image by means of the planimeter. A Zeiss 2 mm. apochromatic objective with no. 4 ocular and tube length of 160 mm. was used in connection with a specially constructed camera of such rigidity as to permit the outlining of fibers on very finely ground glass plates mounted in the plate holder end of the camera. An electric arc consuming 25 amperes of current was used as an illuminant. The optical system was protected from heat by a

water jacketed condenser through which tap water flowed continuously.

By this means we were able to focus upon any fiber and obtain a sharp image which was outlined with pencil on the ground glass at a magnification of 4000 diameters.

Measurements were then taken by the planimeter directly from the glass plate and recorded in square centimeters. Each planimeter measurement was repeated five times to insure accuracy and the result reduced to square micra.

SECTIONAL AREAS OF NERVE FIBERS FROM THE PERONEAL NERVE OF NORMAL ANIMALS

Series no. 3 consisting of three unoperated animals, nos. 222, 223 and 224, of known weights was utilized for these measurements.

To determine the sectional areas of normal nerve fibers, forty of the largest nerve fibers from the proximal end and forty of the largest nerve fibers from the distal end of each specimen were measured. These measurements were then tabulated in the order of their size beginning with the largest fibers. The averages of the first ten records were then taken, the percentage relations of the axis computed and the data arranged as in table 9.

It will be seen from this table that the average of the ten largest fibers in the proximal end of each nerve gives us a measurement of 109.8 square micra for no. 224, 137.7 square micra for no. 223 and 150.3 square micra for no. 222. The average

TABLE 9
Normal rats: Sectional area of fibers; relation of axis to sheath

NO.	WEIGHT	PROXIMAL END				DISTAL END			
		Entire fiber	Axis	Sheath	Per cent of axis	Entire fiber	Axis	Sheath	Per cent of axis
224	104	109.8	55.6	54.1	50.6	85.0	42.3	42.7	49.7
223	117	137.7	75.2	62.5	54.6	85.8	42.6	43.2	49.6
222	182	150.3	82.9	67.4	55.1	113.0	56.7	56.2	50.1
Average.....	135	132.6	71.2	61.3	53.7	94.6	47.2	47.3	49.9

Average sectional area of the proximal and distal ends, 113.6 square micra.

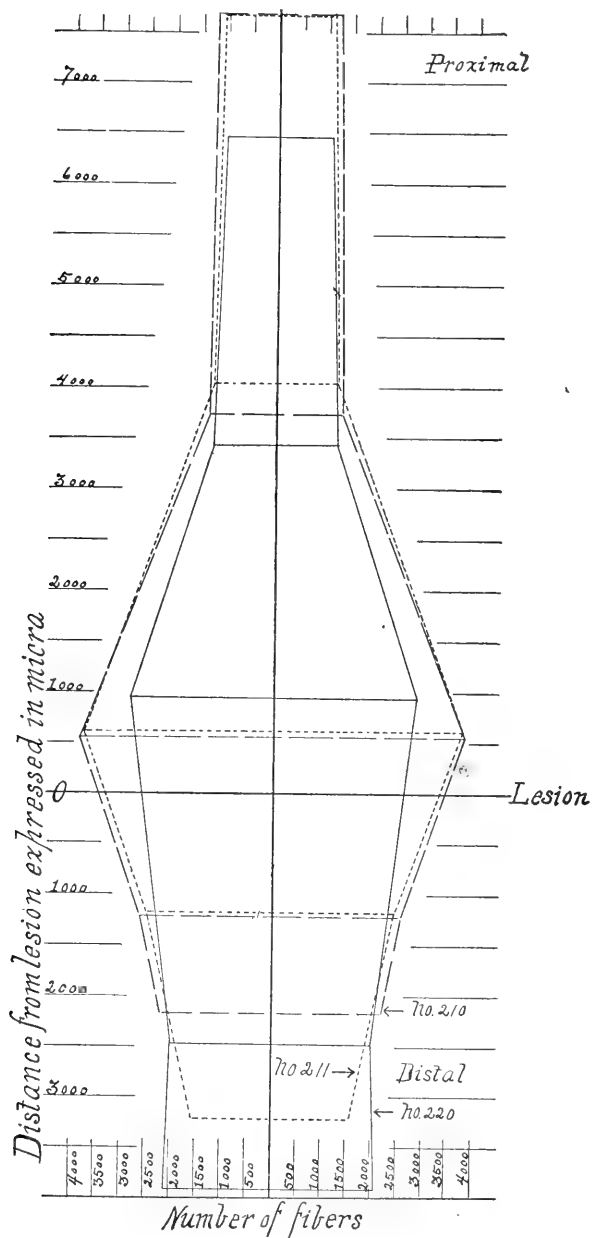


Figure 2

measurements for the ten largest fibers of the distal ends are 85 square micra, 85.8 square micra and 113 square micra respectively. We thus observe that the size of fibers diminishes from the proximal to the distal ends, this diminution averaging about 29 per cent of the proximal measurements, in a distance of 10 mm.—the length of nerve excised. Combining these averages of the proximal and distal ends, we have 113.6 square micra as the average sectional area for the ten largest fibers in the middle zone of the nerve.

We observe also from table 9, that the sectional area of fibers increases as the weight of the animal increases. Taking weight as an index of age we may infer that in young animals nerve fibers increase in sectional area with the age of the animal.

SECTIONAL AREAS OF NERVE FIBERS FROM THE CONTROL SIDE OF OPERATED ANIMALS

Series 7, including operated animals nos. 106, 114 and 154, was utilized for the determination of sectional areas of the fibers of the control nerves and the sectional areas of fibers of the proximal and distal ends of the operated nerves.

Forty or more of the largest fibers from each control nerve and from each proximal and each distal end of each operated nerve were measured. The measurements were tabulated under their proper headings in the order of their size beginning with the largest fibers. From this tabulation the averages of each successive group of ten fibers, beginning with the largest, were taken, the percentage value of the axis was determined for each group and the data arranged in table 10.

From the table we note that the average for the control fibers in group 1 is 65.7 square micra. The average weight of the three animals is 156 grams and their average age is 189 days. From table 9, we find that the average sectional area of the proximal and distal ends of the 10 largest fibers of three normal animals, of an average weight of 135 grams, is 113.6 square micra or an excess of 47.9 square micra over the controls of the

operated animals. We conclude, therefore, that in this instance the operation has reduced the sectional area of the fibers of the control nerve about 42 per cent. This result is all the more striking when we recall that the operated animals are markedly heavier than the unoperated and might be expected therefore to have larger nerve fibers.

TABLE 10
Measurements in square micra

	NO. 106; AGE, 276; WEIGHT, 148; TIME ELAPSED, 26 DAYS			NO. 114; AGE, 164; WEIGHT, 157; TIME ELAPSED, 105 DAYS			NO. 154; AGE, 129; WEIGHT, 162.5; TIME ELAPSED, 98 DAYS		
	Control nerve	Operated nerve		Control nerve	Operated nerve		Control nerve	Operated nerve	
		Prox.	Distal		Prox.	Distal		Prox.	Distal
Group 1: area.....	65.5	57.0	20.7	73.8	51.5	34.5	57.9	58.9	34.5
Per cent of axis.....	47.5	46.1	52.1	40.6	33.0	46.9	44.2	39.7	42.8
Group 2: area.....	53.7	31.4	16.0	63.9	33.0	25.5	47.4	45.7	28.3
Per cent of axis.....	44.8	52.8	50.6	41.4	28.0	48.6	44.5	38.7	39.5
Group 3: area.....	44.5	23.0	10.4	44.9	22.0	18.4	32.5	39.5	25.3
Per cent of axis.....	48.0	50.8	48.0	40.0	32.2	50.0	44.3	39.0	43.0
Group 4: area.....	34.1	12.8	6.8	15.6	10.8		20.3	31.5	22.6
Per cent of axis.....	45.4	46.8	41.1	48.0	38.0		43.5	39.3	39.8
Group 5: area.....	25.3			7.9	6.4		10.4	23.1	18.1
Per cent of axis.....	47.0			58.2	42.0		48.0	41.1	41.9
Group 6: area.....	17.8							19.3	14.8
Per cent of axis.....	47.1							41.4	43.2
Group 7: area.....								14.9	11.8
Per cent of axis.....								38.9	37.2

Average weight of the three animals, 156 grams

Average age of the three animals, 189 days

Average of control nerve in group 1, 65.7 square micra

Average of proximal measurements of group 1, 55.8 square micra

Average of distal measurements of group 1, 29.9 square micra

SECTIONAL AREAS OF FIBERS FROM THE OPERATED NERVE

We note in table 10, that the average sectional areas of the fibers of both the proximal and the distal ends of operated nerves are much less than the average sectional area of the fibers of the control nerves. Considering only the data given in group 1, we note that the average for the fibers from the proximal ends of the operated nerves is 55.8 square micra or 15 per cent less than the average for the fibers of the control nerves. Sections from the controls were taken midway in the course of the nerve and hence should be expected to show smaller fibers than those at the proximal end of the operated nerve.

Also, the average of the fibers of the distal ends of the operated nerves is 29.9 square micra or 54 per cent less than the average of the fibers of the control nerves. These last, however, are newly formed fibers and hence form a class different from either the control fibers or the proximal operated.

From table 10, we note that no. 106 was an adult animal (276 days of age) which was killed 26 days after the operation. Its control fibers average 65.5 square micra, its proximal fibers 57 square micra and its distal fibers 20.7 square micra.

TABLE 11

NO.	AGE AT TIME OF KILLING	TIME ELAPSED BETWEEN OPERATION AND KILLING	AVERAGE OF CONTROL FIBERS IN SQUARE MICRA	AVERAGE OF PROXIMAL FIBERS IN SQUARE MICRA	AVERAGE OF DISTAL FIBERS IN SQUARE MICRA
154	129	98	57.9	58.9	34.5
106	276	26	65.5	57.0	20.7

No. 154 was a much younger animal (129 days of age) and was killed 98 days after operation. Its control fibers average 57.9 square micra, its proximal fibers 58.9 square micra and its distal fibers 34.5 square micra. Placing these data in tabular form for convenience of comparison, we have table 11.

It is evident that no. 154, the younger animal, after a greater lapse of time (98 days) has been able to repair more completely the fibers in the proximal segment of the operated nerve and to regenerate in the distal segment fibers more nearly equal in

sectional area to its control fibers than no. 106, the older animal, surviving only 26 days after the operation.

The objection may be made that the greater lapse of time in the case of no. 154 accounts for its greater development in size of fibers. This objection does not hold good if we compare nos. 114 and 154 in which the lapse of time is greater in the case of the older animal yet it fails to develop fibers as nearly equal to its control as no. 154, the younger animal.

AXIS-SHEATH AREA RELATION: NORMAL ANIMALS

For determining the area relations of axis and sheath, series of measurements were made on fibers from normal animals, on fibers from the control nerve of operated animals and on fibers from the proximal and the distal ends of operated nerves.

A drawing of the magnified image of the fiber section was made outlining the entire fiber and the contained axis cylinder. The area of the entire fiber was first measured by the planimeter, the area of the axis cylinder was then measured and the area of the sheath computed.

Referring to table 9, we see that the average sectional area of axis at the proximal end of the nerve in three normal animals is 71.2 square micra; the average sectional area of sheath is 61.3 square micra. The axis therefore constitutes 53.7 per cent of the fiber. Fibers of the distal end show a slightly different relation. The average of the distal axes is 47.2 square micra, the average of the distal sheaths is 47.3 square micra. The axis here constitutes 49.9 per cent of the fiber. These data show that while both the axis and the sheath taper from the proximal to the distal end of the nerve, yet the axis constitutes a slightly less percentage of the fiber at the distal end, that is, the proportion of sheath has increased at the distal end.

Combining the percentage values of both proximal and distal ends, we obtain the following average percentage for the area relation of axis to sheath:

Axis 51.8 per cent	Sheath 48.2 per cent
--------------------	----------------------

for normal unoperated animals of 135 grams average weight.

Donaldson and Hoke ('05) in an examination of the nerve fibers from a large series of vertebrates which included the albino rat, found the area relation of axis to sheath to be approximately as one to one. The results here given are in substantial agreement with their observations.

Dunn ('12) found in the albino rat that medullated nerve fibers and their axis cylinders increase continuously in size until 270 days of age and that in old rats, about 640 days of age, there is a noticeable decrease in size of both the nerve fiber and its axis cylinder. Dr. Dunn also followed the changes in the axis sheath relations with age.

AXIS-SHEATH AREA RELATION: OPERATED ANIMALS

Passing to the consideration of the axis-sheath area relation in operated animals, we observe from table 10 that the percentage of axis in operated animals is less than in normal animals. The average percentage value of the axis in the largest fibers of the controls shown in group 1 (nos. 106, 114 and 154) is 44.1 per cent. The sheath, therefore, constitutes 55.9 per cent, but it should be recalled that these fibers in the control nerve have suffered a diminution in total area of 42 per cent as the result of the operation.

If we take from table 10 the average percentage value of the axis, and compute the sheath value, (1) of the controls and (2) of the proximal, and (3) distal ends of the operated nerves in the first four groups, and then arrange these values under their proper headings, we get table 12.

TABLE 12

NO.	AGE	TIME ELAPSED	CONTROL NERVE		OPERATED NERVE			
					Proximal		Distal	
			Per cent of axis	Per cent of sheath	Per cent of axis	Per cent of sheath	Per cent of axis	Per cent of sheath
106	276	26	46.4	53.6	49.1	50.9	47.9	52.1
114	164	105	42.5	57.5	32.8	67.2	48.5	51.5
154	129	98	44.1	55.9	39.2	60.8	41.2	58.8
Average.....			44.3	55.7	40.3	59.7	45.8	54.2

From this summary of axis values, we observe that the axes in all controls of operated animals have been reduced below that of a normal fiber, the average for the three animals being 44.3 per cent, the percentage of sheath being correspondingly increased.

In examining the percentage relations of the operated nerves as shown in table 12, it is evident that in the loss in size of fibers of operated nerves the reduction in the percentage of axis is more marked in the animals which are younger and which have lived longer periods since the operation as nos. 114 and 154. This would accord with Dunn's observations ('12).

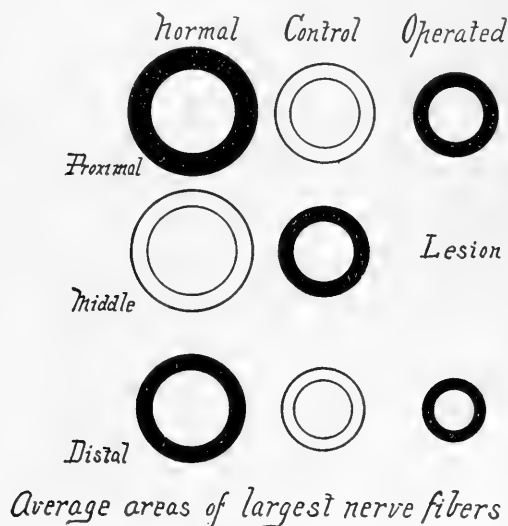


Figure 3

The control nerve of no. 106 varies less in its axis-sheath relation from the normal than the control nerve of no. 114, a younger animal. It will be noted also that the deviation from normal relations in the distal ends of operated nerves is less marked than in the proximal ends, but the distal ends are newly formed while the proximal ends are simply showing modifications. The greater reduction in percentage relation of the axis in the proximal segments of operated nerves as compared with the relation in the

control, may be due to the branching which takes place in the proximal zone when new fibers are produced at the expense of the parent stem.

In order to bring together the general results of the observations on the relations of the area of the axis and of the sheath, figure 3 has been constructed. In this figure the total areas and the axis sheath relations of the averages from the ten largest fibers taken respectively from the normal, control and operated nerves are given. In each instance the area of the fibers at the proximal end, the middle zone and the distal end are drawn. Where the determination depends on direct observation, the medullary sheath is shown as a black ring, where the area has been computed the outlines of both sheath and axis are indicated by lines. The size of the fibers thus represented serves to show more clearly, than the simple measurements can, the striking modifications of the fibers which occur in the operated animals.

LITERATURE

The literature on the subject of regeneration has been carefully reviewed by Ranson (*Jour. Comp. Neur.*, vol. 22, no. 6, 1912). We will, therefore, call attention only to those papers dealing directly with the number of fibers (including branching), their size and the axis-sheath relation in the peripheral nervous system of vertebrates.

Rudolph Wagner ('47) observed that in the distribution of primitive fibers in the electric organ of fishes there is true branching and that one primitive fiber may have as many as twenty-five branches. He demonstrated that the branching of nerve fibers was a true branching and not the formation of a network.

Schwalbe ('82) studied the size of fibers and the relation of length to diameter. He attempted to show that the larger fibers were distributed to the most distant parts. This was disproven by Dunn ('02) in her study of the nerve fibers of the frog's leg. Dunn here found a conical diminution in the nerve fiber in its course.

Voischvillo ('83) studied the numerical relations of sensory fibers to the skin of the extremities and of motor fibers to muscles which move rapidly and those which move less rapidly but with great force. He found that the skin of the upper extremities was more abundantly supplied with sensory fibers than the skin of the lower extremities, also that the eye muscle received a greater proportion of nerve fibers to muscle fibers than the muscles of the extremities.

Vashkevitch ('87) determined the number of fibers in the n. ischiadicus and n. medianus of bats, mice, rats, marmots, rabbits, cats and dogs and found that the absolute number of nerve fibers depended upon the weight of the central nervous system and the weight of the body, but that the increase in number of fibers does not progress at the same ratio with the increase in body weight.

Fritsch ('89) determined, in the torpedo the numerical relations of the elements of the electric organ to the nerve cells and nerve fibers.

Dunn ('00) observed that practical equality exists in the number of fibers in the legs of the two sides of the frog.

Dunn also ('02) showed by numerical determinations on the nerve distribution in the frog's leg that the number of fibers normally increases by branching as one passes distally.

Hatai ('02) in a study of the ganglion cells and the dorsal root fibers determined the ratio of nerve fibers to cells in the white rat at 10.3 grams body weight and at maturity.

Hatai ('03) also found that the rate of increase in the number of fibers of the ventral roots of the spinal nerves of the albino rat was most rapid between the body weights of 10.3 and 25.4 grams and that the number found at maturity is 2.7 times that found in the 10.3 gram rat.

Donaldson ('03) in considering the number of fibers distributed to the skin and muscles of the frog's leg found that this distribution followed a fixed law expressed as follows: The nerve fibers entering the leg of the frog (*Rana virescens*) by the sciatic and crural nerves, are distributed to the thigh, shank and foot

in numbers which, for each of these segments are equal to the sum of the efferent fibers—taken in proportion to the weight of the muscles—and of the efferent fibers—taken in proportion to the area of the skin.

Ingbert ('04) studied the areas of cross section in the ventral and dorsal roots of the spinal nerves and computed the number of sensory and motor fibers and showed that during the increase in the nerve supply the gain has been more in the sensory than the motor fibers.

Donaldson and Hoke ('05) in a series of observations on the spinal nerves of a number of vertebrates found that the relation of axis cylinder to sheath was approximately as one to one.

Boughton's ('06) results on the albino rat and on the cat correspond with ours in finding an increase in the number of fibers and an increase in size of fibers as the animal increases in weight. He also points out that fibers which develop after the period of rapid growth never attain large size.

Perroncito ('06) showed that an axon begins to regenerate by sprouting from the proximal stump within three hours after section, and that in many cases a single fiber gives rise to many sprouts.

Osborne and Kilvington ('08) proved that bifurcation of motor axons took place when a plurality of path was offered and that motor axons would bifurcate and follow a sensory path as well as a motor path. In a later paper ('09) they supplement their results by further observations and state that bifurcation occurs to some extent at the point of section as well as at the point where plurality of paths is offered.

Dunn ('09) in a paper on the albino rat observes the splitting of nerve fibers and notes the deviation from the one to one relation in the area of sheath and axis according to age. In a later paper ('11) she observes the loss of fibers in old animals.

Ranson ('12) observed the branching of medullated nerve fibers 5 mm. above the section.

SUMMARY OF RESULTS

1. The peroneal nerve of the normal albino rat of 135 grams body weight was found to contain 2288 medullated fibers in its proximal end and 2323 medullated fibers in its distal end. The middle zone is estimated to contain 2306 fibers. The portion of the nerve utilized for these experiments was 10 mm. long.

2. There is an increase in the number of fibers as we pass from the proximal to the distal end of this 10 mm. of nerve amounting to 1.5 per cent of the proximal number.

3. The number of fibers is approximately the same for each side.

4. The number of medullated fibers increases with body weight (= age) during the first 276 days of life. The increase between the 128-129-day group and the 276-day group (table 4) is about 5 per cent.

5. Crushing the nerve by the method used causes complete degeneration beyond the point of the lesion.

6. Four days after the operation no normal fibers are to be found on the distal side of the lesion. The degeneration is assumed to have involved the entire distal portions of the fibers.

7. Complete degeneration also extends from 2 to 3.2 mm. on the proximal side of the lesion.

8. Characteristic loss of motor control always follows the operation. In many cases this has seemingly disappeared at the end of ten days, probably as the result of compensatory adjustment.

9. The general effects of the operation are more pronounced on older animals.

10. The control nerve of an operated animal contains fewer medullated fibers than the same nerve from a normal animal of the same age. This loss in number is one of the effects of the operation, and in the cases here examined amounted to 16 per cent.

11. Following the degeneration in the operated nerve, regeneration, accompanied by branching of axons, takes place and there is an increase of from 64 to 249 per cent in the number of medullated fibers on the proximal side of the lesion.

12. The increase in number of fibers on the distal side of the lesion is less than on the proximal side but the number always exceeds that found on the control side.

13. The number of regenerated fibers rapidly increases as the region of the lesion is approached from the proximal side; the number decreases as we pass from the lesion distally. Over 13 per cent of the regenerated fibers arise from a point more than 7 mm. above the lesion.

14. The average sectional area of the 10 largest fibers in the middle zone of the peroneal nerve of a normal albino rat of 135 grams body weight was found to be 113.6 square micra.

15. The nerve fiber tapers from its proximal to its distal end (see table 9). The sectional area of fibers increases with the age of the animal during the first 276 days of life.

16. The average sectional area of the 10 largest fibers from the control nerve of an operated albino rat of 156 grams body weight is 65.7 square micra.

17. One of the results of operation is, therefore, a loss in sectional area of nerve fibers of the control nerve. In this instance the loss amounts to 42 per cent. It is possible that this effect is general throughout the peripheral nervous system.

18. The sectional area of the 10 largest nerve fibers on the proximal side of the lesion is 55.8 square micra, or 15 per cent less than the area of the fibers of the control nerve.

19. The sectional area of the 10 largest regenerated nerve fibers on the distal side of the lesion is 29.9 square micra or 54 per cent less than the area of the fibers in the control nerve.

20. In the normal albino rat of 135 grams body weight, the axis-sheath relations of the fibers of the peroneal nerve are as follows: Area of axis 51.8 per cent. Area of sheath 48.2 per cent.

21. In a series of three operated animals of an average weight of 156 grams, the average axis-sheath area relation is as follows:

Controls.....	Axis 44.3 per cent	Sheath 55.7 per cent
Proximal end of operated		
nerve.....	Axis 40.3 per cent	Sheath 59.7 per cent
Distal end of operated		
nerve.....	Axis 45.8 per cent	Sheath 54.2 per cent

22. Thus in the operated animal in which the fibers of both the control and operated nerves are all diminished in total area, the axis-sheath relation is such that in all three localities the area of the axis is relatively less than in the fibers from the normal animal.

23. This deviation from the normal in the case of the control nerves and the proximal end of the operated nerves represents an alteration in existing structures while in the fibers distal to the lesion, it appears in structures which have been newly formed.

CONCLUSIONS

The relation of the more important results here given to previous information is as follows:

The observations that medullated nerve fibers branch in their course and in a given nerve increase in number for a time, with age, is in accord with the findings of all the authors who have studied these matters. Also, in agreement with others is the fact that the number of fibers on the two sides of the same animal is similar. In agreement with Schwalbe ('82) and Dunn ('02) it is found that the largest fibers in the peroneal nerve undergo a conical diminution.

The fact that in the operated animals the number of fibers is diminished on the control side has been reported by Dunn ('09) for the frog. The observations that the fibers of the control side are greatly diminished in diameter and that the area relation of the axis and sheath is modified—also that the same is true for the fibers in the proximal portion of the operated nerve, are all new.

The results on the area relation of the axis-sheath in the fibers of the normal nerve agree in general with the observations of Donaldson and Hoke ('05), but the determinations of this relation in the newly formed fibers on the distal side of the lesion has not been previously made.

The observations of Perroncito ('06), Osborne and Kilvington ('08-'09) and the study of neuromata all indicate a tendency to branching in the regenerating fiber. Our observations give precise information as to the amount of this branching, the general

distribution of the fibers in the nerve near the lesion and also show that the increase in number begins high up in the course of the proximal portion of the nerve. The fate of these excess fibers has not yet been determined.

The most important outcome of this investigation is the evidence it furnishes of an alteration in the number and size of the fibers in the control nerve and in the proximal end of the operated as well as the accompanying changes in the diameter of the fibers and the area relation of the axis-sheath.

These alterations, as they stand, apply to symmetrical peripheral nerves, but it seems possible that they are more widely distributed. The sensitiveness of the area relation of the axis-sheath to disturbance of the normal conditions in the animal points to a high degree of nutritive response in the medullated fiber.

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A COMPARATIVE STUDY OF THE BRAINS OF THREE GENERA OF ANTS, WITH SPECIAL REFERENCE TO THE MUSHROOM BODIES

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FORTY-TWO FIGURES

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INTRODUCTION

This subject was suggested to me by Prof. W. M. Wheeler of Harvard University as one that needed investigation. After my work was begun, Pietschker ('11) published his paper "Das Gehirn der Ameise." Although I can corroborate much of Pietschker's work, I differ from him in several important points. Professor Wheeler has told me that Pietschker's *Camponotus ligniperdis* is, in regard to internal structure, one and the same as the *Camponotus pennsylvanicus* of this paper, the two forms differing only in very slight external characters. I am greatly indebted to Professor Wheeler for his interest in my work, for the identification of specimens, and for material from his collections which will be used in a second paper on the brains of other genera of ants.

MATERIAL AND METHODS

The genera and species described in this paper are: *Camponotus herculeanus* L., subsp. *pennsylvanicus*, *Formica pallidefulva*, Latreille, subsp. *Schaufussi*, *Lasius niger* L., var. *americana*.

The material was collected in 1911 and 1912 during June and July, at which time the developing stages are very abundant. *Camponotus* colonies were found by chopping open partly decayed logs, *Formica* and *Lasius* were found in the ground under stones or logs. Following the example of Wheeler ('10) and Pietschker ('11) the pupa was the only stage used in this work, for at this period the skin is so soft and free from chitin that the entire head and body can be sectioned. The brain of the oldest pupae differs very little from that of the adult, and the difficulty of dissecting out this delicate organ is avoided. Both very young and nearly mature pupae were used, the approximate age being determined by the pigmentation of the eyes. The youngest pupae have colorless eyes, which later become pinkish, reddish brown, and finally black. The pupae are enveloped in tough brown cocoons which were removed with fine needles under a dissecting lens. The heads were then detached from the bodies and dropped into the fixative. The size of the cocoons

depends upon the caste, and after a little practice one can sort the different castes before opening the cocoons. If the collected material was too young to use it was found possible to keep the larvae in artificial nests with a number of workers. In every case the young were moved about and cared for by the workers and developed in good condition.

The fixatives used were Gilson's fluid, very good for both nervous and other tissues and especially good for the fiber bundles of the brain; Flemming's fluid, good for nerve cells though not so good for nerve fibers; 10 per cent formalin, very poor, tending to soften and disintegrate the tissues; Kalil's fluid, very good. Cajal's method, used by Jonescu ('09) for the honey bee (namely, silver nitrate in the dark at 30°C., followed by pyrogallie acid), was tried but without success.

The material for sectioning was embedded in paraffin from three to six hours, the sections were cut 6 μ thick and stained on the slide. The most successful stain was Ehrlich's acid hematoxylin, followed by ammonia alcohol, and counterstained either with eosin or Orange G. Iron hematoxylin is especially good for differentiating the fiber bundles of the mushroom bodies. Whole mounts of the heads were made by staining in Conklin's picro-hematoxylin for a long time, twelve to forty-eight hours, the longer time in the case of large heads like the *Camponotus* queens and soldiers, and then destaining in 70 per cent acid alcohol from six to twenty-four hours. After being dehydrated and cleared in cedar oil the heads were mounted in damar, frontal side up. When successfully destained all parts of the brain, the nerves, the ocelli, and some of the glands of the head may be clearly seen. The one disadvantage of this method is that it is not permanent, the preparations fading after a few months, especially those that have had the prolonged treatment in acid alcohol.

Borax carmine has also been used for whole mounts of the heads, but although the preparations are permanent, they are less transparent and show less than the hematoxylin mounts.

THE ANT BRAIN

1. HISTORICAL ACCOUNT

The older writers conceived of the insect brain as consisting of six parts, corresponding to the six embryonic segments of the head: I, the acron; II, the antennary segment; III, the intercalary segment, all preoral, and constituting the supraesophageal part of the brain; IV, V, VI, the mandibular, maxillary and labial segments, postoral, and forming the subesophageal mass. Owing to the tendency toward a fusion of parts and to the changes in position that have occurred the homologies of some of these segments are difficult to determine, and wide differences of opinion have resulted, but the later researches, especially those of Janet ('05) and Jonescu ('09) tend to establish the older view on a firmer basis.

Viallanes ('86) described the insect brain as consisting of the following parts: Segment I, the protocerebrum, including the protocerebral lobes, optic lobes, mushroom bodies, and ocelli; segment II, the deutocerebrum, consisting of the antennary lobes; segment III, the tritocerebrum, the nerves of the labrum. The tritocerebral nerve of Viallanes was therefore the labral nerve. Segments IV, V and VI formed the subesophageal part of the brain.

Haller ('04) believed in a very different homology of the head region, based on a comparison of the insect, with the myriapod head, but his view seems quite untenable.

Janet ('05) although advocating the division of the brain into six parts, differed from Viallanes in believing, first, that the labro-frontal nerve, which now arises posterior to the antennary nerves, should be considered as belonging to the protocerebrum and as arising originally in a more anterior position; second, that the tritocerebral nerve is represented by the nerve which supplies the interior dilator muscle of the pharynx in *Lasius niger*. This is a small unpaired nerve, running beneath the pharynx and arising from two roots posterior to the lateral nerves. The very small lobes from which the paired roots arise represent all that is left of the tritocerebrum or third head segment.

The work of Janet has been confirmed by Jonescu ('09) who, in the honey bee, finds in a similar position the nerve supplying the inferior dilator muscle of the pharynx, and demonstrates the origin of its two roots in two small fibrous masses and groups of ganglion cells which he regards as the reduced tritocerebral lobes (Jonescu '09, figs. 41-42).

Table 1 gives the parts which may be distinguished in a typical ant brain.

TABLE 1

The parts of the brain

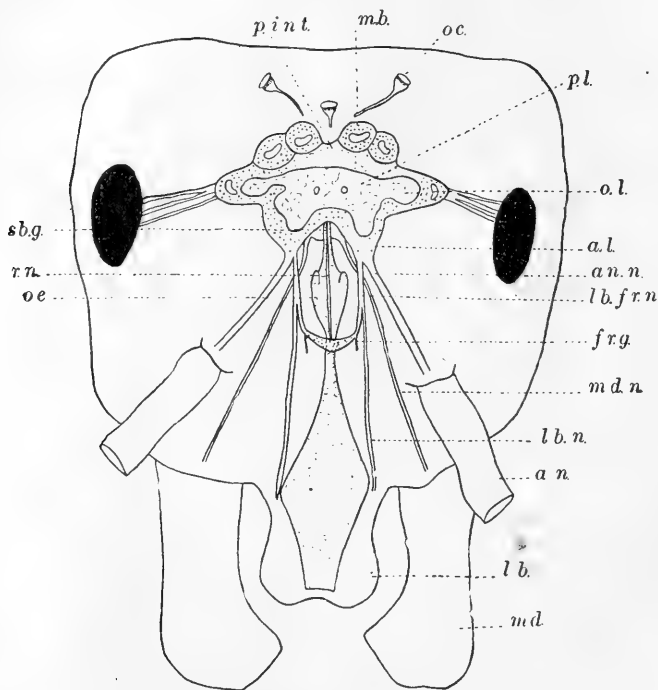
- I. The supraesophageal ganglion
 - A. Protocerebrum
 - The protocerebral lobes
 - The protocerebral commissures { 1. anterior, dorsal
2. posterior, dorsal
 - The optic bodies
 - The optic lobes
 - The ocellar lobes
 - The central body
 - The mushroom bodies { anterior roots
central body roots
posterior roots
 - B. Deutocerebrum
 - The antennal lobes
 - C. Tritocerebrum.
 - The tritocerebral ganglia
- II. The subesophageal ganglion
 - The ventral connectives
 - The mandibular } ganglion
 - The maxillary }
 - The labial }

The nerves of the head

- The optic nerves
- The ocellar nerves
- The antennal nerves
- The labro-frontal nerves { the labral nerves
the frontal nerves to the frontal ganglion
- The recurrent nerve, and other smaller nerves arising from the frontal ganglion
- The tritocerebral nerve (inferior dilator of pharynx)
- The mandibular nerves
- The maxillary nerves
- The labial nerves
- The accessory nerves (?)
- The salivary nerves

2. GENERAL ANATOMY

Text figure 1, a diagram drawn from a semitransparent mount of the whole head of the queen of *Camponotus pennsylvanicus*, shows the following parts: (1) the supraesophageal ganglion, above the esophagus, its first part, the protocerebrum, consist-



Text fig. 1 The head of the queen of *Camponotus pennsylvanicus*. *An.* antennae; *a.l.*, antennary lobes; *an.n.*, antennary nerves; *fr.g.*, frontal ganglion; *lb.fr.n.*, labrofrontal nerve; *lb.n.*, labral nerve; *lb.*, labrum; *m.b.*, mushroom body; *md.*, mandible; *md.n.*, mandibular nerve; *oe.*, oesophagus; *o.l.*, optic lobe; *oc.*, ocellus; *p.int.*, pars intercerebralis; *p.l.*, protocerebral lobe; *r.n.*, recurrent nerve; *sb.g.*, subesophageal ganglion.

ing of the protocerebral lobes, *p.l.*, which form the greater part of the brain, the optic lobes, *o.l.*, lateral extensions of the protocerebral lobes and connected with the compound eyes by the numerous bundles of optic nerve fibers, the three ocelli, *oc.*, connected by the ocellar nerves, with the ocellar lobes within the

brain, and the mushroom bodies, *m.b.*, prominent bilobed structures forming the apex of the brain. Each mushroom body consists of an outer zone of nerve cells surrounding the calyces, two deeply indented cup-shaped masses of nerve fibers, which, in the whole mounts of the head, appear as two stout crescents. The fibers of the calyces continue downward into the protocerebral lobes as the stalks of the mushroom bodies, each stalk branching into the anterior root and the posterior root. The paired labro-frontal nerves, *lb.fr.n.*, which arise each from a single root posterior to the origin of the antennary nerves, soon divide into two branches, the labral nerve, *lb.n.*, to the labrum, and the frontal nerve, running forward to the frontal ganglion, *fr.g.*, situated between the bases of the antennae near the anterior surface of the head. Several small nerves, whose course was not determined, run forward from this ganglion; the recurrent nerve, *r.n.*, also arises in it, but runs backward above the esophagus throughout its length. The second part of the esophageal ganglion, the deutocerebrum, consists of the antennary or olfactory lobes, *a.l.*, ventral extensions of the protocerebral lobes, ending in the nerve trunks going to the antennae. The third part of the supraesophageal ganglion, the tritocerebrum, is hidden by the esophagus and protocerebrum, but its nerve, the tritocerebral nerve, *tr.n.*, may be seen beneath the esophagus going to the inferior dilator muscle of the pharynx. (2) the subesophageal ganglion, *sb.g.*, is seen ventral to the supraesophageal mass, and from it arise the paired nerves that supply the mouth parts and the salivary glands.

COMPARISON OF THE BRAINS OF THE CASTES

1. *The queen brain*

a. Camponotus queen: figure 1; text figure 1. The brain of the *Camponotus* queen is about one-third of the width of the head, and is situated farther away from the dorsal surface or apex of the head than in either the worker or the male, figures 2 and 3. All parts are proportionately large except the mushroom bodies, *m.b.*, these, on the contrary, are relatively small, projecting very little on the dorsal surface of the brain, and lying quite widely

separated from each other and from the median line. The optic lobes, *o.l.*, are the most highly developed part of this brain, and extend far out in each lateral direction. These two points of structure, namely, the slight development of the mushroom bodies and the lateral extension of the optic lobes, give this brain a flattened, laterally drawn out appearance which is very characteristic. The compound eyes and the ocelli are very large, the former slightly smaller, the latter larger than those of the male.

b. Formica queen: figure 4. The brain occupies more than one-half the width of the head, and is nearer to the apex than in *Camponotus*. The optic lobes, *o.l.*, are large, but are relatively less extended in the lateral direction and more extended in the dorso-ventral direction. The mushroom bodies, *m.b.*, are not highly developed, although they project farther on the dorsal surface and extend nearer to one another. Both compound eyes and ocelli are smaller than those of the male *Formica* (fig. 6).

c. Lasius queen: figure 7. The brain occupies more than three-fourths of the width of the head. Taking the mushroom bodies and the optic lobes as a standard, this brain is more highly developed than that of any other queen or of any other caste. By measurement the mushroom bodies of the *Lasius* queen are larger in proportion to the total size of the brain than those of *Camponotus* or *Formica*. By comparing figures 1, 4 and 7 it will be noted that these bodies extend farther in a dorsal direction and nearer to one another in this genus. Furthermore, the mushroom bodies of the *Lasius* queen are almost equal in size, if not actually equal, to those of the *Lasius* worker (fig. 8). Measured from side to side the queen mushroom bodies are wider than those of the worker, measured in the dorso-ventral direction the worker has a slight advantage over the queen. The optic lobes, *o.l.*, have the same lateral extension as in *Camponotus*, but the dorso-ventral thickness is less than in *Formica*. The compound eyes are larger than those of the male, the ocelli smaller.

To summarize: in these three genera the queen has the largest head and the largest brain. The brain is usually extended in the lateral direction owing to the size and the lateral position of the optic lobes. The mushroom bodies may be equal, or nearly

equal, in size to those of the worker, *Lasius*, or smaller than in the worker, *Camponotus*, *Formica*. Compared with the male, the queen's compound eyes are usually slightly smaller, the ocelli, either larger, *Camponotus*, or smaller, *Formica*, *Lasius*.

2. *The worker brain*

a. Camponotus worker: figure 2. The brain occupies about two-thirds of the width of the head. It is nearer the apex of the head than in the queen but farther away than in the male. The distinguishing characters of this brain are its highly developed mushroom bodies, *m.b.*, and its greatly reduced optic lobes, *o.l.* The mushroom bodies make two huge swellings on the dorsal surface, almost touching one another in the median line and extending far out in the lateral direction. The cups are more deeply lobed than in any other caste and the large swollen ends of the crescents make these bodies very prominent. The optic lobes are reduced in both directions. This reduction is evidently correlated with the reduced size of the compound eyes. Ocelli are entirely absent in this worker.

b. Formica worker: figure 5. The brain occupies more than two-thirds of the width of the head and lies at about the same distance from the apex as in the other two castes of this genus. Although the heads of the worker and queen are about similar in size the worker brain is actually larger than that of the queen. Both mushroom bodies, *m.b.*, and optic lobes, *o.l.*, are much larger and the optic lobes are thicker and curve a little downward toward the large well developed compound eyes, as in a male brain. The mushroom bodies project prominently on the dorsal surface, but do not approach each other as much as in the *Camponotus* worker. The ocelli are reduced in size.

c. Lasius worker: figure 8. The brain of this form occupies nearly the whole width of the head. The reduced size of the optic lobes, *o.l.*, and the large well developed mushroom bodies, *m.b.*, are characteristic. As stated above, the mushroom bodies are equal to or slightly larger than those of the queen. Like the worker of *Camponotus*, the reduction of the optic lobes is

correlated with that of the compound eyes, which are much smaller than in the queen and male. The ocelli are very minute, but may be distinguished in sections.

To summarize: the workers of *Camponotus* and *Lasius* have greatly reduced optic lobes, correlated with the smaller compound eyes. *Formica schaufussi* with large compound eyes has large optic lobes, but *F. fusca* with somewhat reduced compound eyes has a reduction in the optic lobes. All these genera have large mushroom bodies, equal or nearly equal to those of the queen, *Lasius*, larger than those of the queen, *Camponotus*, *Formica*. The ocelli are either much reduced, *Formica*, *Lasius*, or absent, *Camponotus*.

3. The male brain

Camponotus, *Formica*, *Lasius*, figures 3, 6 and 9. The brains of the males of these three genera are so similar that one general description will apply to all. This is evidently the least variable, conservative caste.

The head of the male is the smallest with usually the largest compound eyes and large ocelli. The brain occupies nearly the whole width of the head and at least half of its interior. The distinguishing characters of the male brain are the very highly developed optic lobes, *o.l.*, stout, and curving down toward the large compound eyes, and the relatively well developed mushroom bodies, *m.b.* These, although actually smaller than in the other castes, are relatively as large, or nearly as large, in proportion to the size of the heads and to the bulk of the brain.

THE FINER STRUCTURE OF THE BRAIN

1. THE BRAIN SHEATH

The entire brain is surrounded by a delicate sheath composed of small nerve cells placed side by side (fig. 30). These cells are elongated and form a layer appearing like a columnar epithelium. The cytoplasm of the upper or outer surface is dense and takes a deeper stain, giving almost the appearance of a cuticle. The proximal, inner, ends are prolonged into delicate fibers which form a network, separating the sheath cells from the inner ganglion cells which they cover.

2. THE PROTOCEREBRAL LOBES

The protocerebral lobes, "les lobes protocérébraux" of Viallanes ('86), consist of a central core of fibrous reticular substance, surrounded by an outer zone of nerve cells (figs. 1-9). The fibrous substance is composed of: (1) nonmedullated nerve fibers, axons, usually possessing a neurilemma or nucleated sheath, (2) the branching dendrites of the nerve cells, and (3) of supporting cells. The nerve cells that immediately surround the fibrous core of the protocerebral lobes, usually in a single layer, are very small with very little cytoplasm. The outermost cells are large, some very large, with more abundant cytoplasm and large round nuclei. These cells are usually arranged in bunches or masses several cells deep, with their axons running inward to the fibrous core. The median dorsal region, both in front of and beneath the mushroom bodies, called by Haller "the pars intercerebralis," or intercerebral region, is characterized by a looser, more open appearance and by the presence of numerous very large ganglion cells. One of these cells is shown in figure 31. These are, however, not confined to the dorsal region but are found also on the ventral surface of the brain. Jonescu ('09, p. 141) has noted the same facts in the honey bee:

Die Protocerebralloben sind an allen Seiten von einer dünnen Schicht kleiner Associationszellen umgeben. Ausserhalb dieser Schicht findet man eine Schicht grosser Ganglienzellen (Verbindungselemente) Diese Schicht besitzt auffällig grosse Zellen. Solche Zellen sind aber nicht nur an der dorsalen Seite der Protocerebralloben lokalisiert, sondern ich habe sie auch auf der Ventralseite der Loben gefunden.

To understand the relation of the protocerebral lobes to the other parts of the brain, a series of frontal sections taken at varying distances from one another from the brain of the *Lasius* queen, beginning at the anterior end and going backward are shown in figures 10 to 16. It will be noted in figure 10 that the fibrous core of the protocerebral lobes, *p.l.*, is one single solid mass, penetrated by the anterior roots of the mushroom bodies, *a.r.*, and on the ventral surface extending into two tiny fibrous masses, the optic bodies, *o.b.*, 'tubercules optiques,' Viallanes.

The antennary lobes, *a.l.*, lie ventral to the protocerebral lobes, and some of the antennary nerves issue at this point. The four branches of the ocellar nerves, *o.n.*, are seen outside the brain sheath, above the pars intercerebralis, the space between the mushroom bodies. The esophagus, *oe.*, with the recurrent nerve, *r.n.*, above it, lies between the antennary lobes.

In figure 11 the protocerebral lobes are deeply indented for a short distance, about five sections, and the space is filled with small nerve cells and with axons from large cells in the intercerebral region. The nerves from the ocelli have entered the brain and form the four ocellar lobes, *oc.l.* The anterior parts of the optic lobes have appeared, and the double roots of the labro-frontal nerves, *l.f.n.*, make their exit from the antennary lobes.

Figure 12 shows that the previously solid fibrous core becomes separated in the mid-dorsal region into bundles of fibers running from side to side in front of and between the stalks of the mushroom bodies. These fibers are the anterior part of a commissure which in this paper will be termed the "anterior dorsal commissure, *a.cm.*, and which will be described in more detail under the heading of the protocerebral commissures. The stalks of the mushroom bodies, *st.*, penetrate deep into the fibrous core but never meet in the mid-line, remaining always separated by a narrow space. The optic nerves enter the optic lobes, *o.l.*, both in this and in the preceding section.

Figure 13 shows that the dorsal and median part of the protocerebral lobes has disappeared except for the slender anterior commissure, *a.cm.*, which passes in front of the mushroom body stalks above the central body, *c.b.*, connecting the lateral protocerebral lobes. These are also united by a narrow tract of fibers lying above the esophagus but below the mushroom body stalks. The three parts of the optic lobes, outer, middle, and inner fiber masses, are clearly shown, *o.f.*, *m.f.*, *i.f.*; the ocellar lobes have increased in size and lie near together, the outer lobes, from the lateral ocelli, are larger than the inner lobes, from the anterior ocellus. Fibers connect the central body, *c.b.*, with the lateral protocerebral lobes.

Figure 14 shows that the anterior dorsal commissure, *a.cm.*, is becoming very slender and is about to disappear, which happens in the next section—not the next figure. In the sections between this and the preceding figure (fig. 13), this commissure served as a path for fibers that come from the inner ocellar lobes, which have now disappeared. In this section (fig. 14), fibers from the outer ocellar lobes, *o.oc.l.*, also pass into the commissure. The relation of the ocellar nerve fibers to the protocerebral commissures will be more fully discussed under the headings of the protocerebral commissures and ocellar nerves. The central body is still connected by fibers with the protocerebral lobes. The distal ends of the stalks of the mushroom bodies are seen below the central body, divided into two masses, a dorsal and a ventral, the ventral mass labeled *p.r.* in figure 14, the upper parts of the stalks have disappeared. The approach of the two halves of the brain beneath the esophagus indicates that the subesophageal ganglion is about to appear.

Figure 15 shows the subesophageal ganglion, *sb.g.*, with the mandibular nerves, *md.n.*, issuing from it. The protocerebral lobes merge into the ventral connectives with the subesophageal ganglion, but are still connected with each optic lobe by a slender strand of fibers, and with each other, first, by the fiber tract just above the esophagus, and second, by a commissure which has now appeared, which will be termed in this paper the posterior dorsal commissure, *p.cm.*, and which will be described in detail under the heading of the protocerebral commissures. The so-called "tubercles of the central body," *p.r.*, lie beneath the posterior dorsal commissure.

Figure 16 is a section through the posterior part of the subesophageal ganglion, showing the origin of the labial nerves, *lb.n.*, the tritocerebral lobes, *tr.l.*, and the tritocerebral nerve, *tr.n.*

3. THE PROTOCEREAL COMMISSURES

There are two commissures, or horizontal fiber tracts, present on the dorsal surface of the protocerebral lobes, and connecting the opposite sides. The first, here termed the "anterior dorsal commissure" begins a short distance anterior to the central body, and continues above and nearly to the end of this body (figs. 12,

13, 14 and 17, *a.cm.*). The second, here termed the "posterior dorsal commissure," arises several sections posterior to the termination of the first, and lies behind the central body (figs. 15, 20, 21, 22 and 23, *p.cm.*).

The anterior dorsal commissure

This commissure was termed by Viallanes, "la commissure cérébrale supérieure," by Kenyon ('96), "the superior dorso-cerebral commissure," by Jonescu, "die dorsale Kommissur." It is of considerable thickness in an antero-posterior direction, being present in the brain of the *Lasius* queen in ten sections each 6 μ thick. It arises from the protocerebral fibrous core lateral to the stalks of the mushroom bodies, and as it curves in front of them there is doubtless an interchange of fibers. The connection of this commissure with tract *b* of the mushroom bodies is shown in figures 12, and 32 to 40. Although the anterior dorsal commissure is here described as a unit, careful examination of its structure shows that it is composed of several fiber bundles, or commissures, of different origin, and must be therefore a compound structure. It seems probable that this is an indication of the ladder-like arrangement of fibers seen in many invertebrate brains.

The posterior dorsal commissure

This commissure arises from the dorsal surface of the fibrous core of the posterior portion of the protocerebral lobes (fig. 15, *p.cm.*, and figs. 20-23). The fibers curve upward from the lateral portions of the lobes, then across, and down again into the core of the opposite side. This commissure has a very characteristic form, like a broad inverted letter \cap , and is easily recognized. The so-called "tubercles of the central body" often lie beneath it.

This commissure has been termed by Viallanes, "le pont des lobes protocérébraux," by Kenyon, "the fibrillar arch," and by Jonescu and also by Pietschker, "die Ocellarnervenbrücke." Neither Viallanes' nor Kenyon's terms differentiate between this second, posterior commissure, and the first, anterior commissure, so they have not been used in this paper; Jonescu's term, "Ocel-

larnervenbrücke," although applicable to the honey bee and to some ants, is a misnomer in the case of certain other ants. Figures 14 and 15 show us, first, that in the queen of *Lasius niger*, the fibers of the ocellar nerves pass into the protocerebral lobes together with the last fibers of the first or anterior dorsal commissure; second, that the ocellar lobes have disappeared from the section in which the posterior dorsal commissure begins. It is plain, therefore, that in the queen of *Lasius niger* there is no relation whatever between the ocellar nerves and the posterior commissure. The same is true of the *Camponotus* queen, all the fibers passing into the brain with the last fibers of the anterior commissure, none with the posterior dorsal commissure, which in these two queens is certainly not an "Ocellarnervenbrücke." In the *Formica* queen (fig. 23), the ocellar nerve fibers do not pass to the brain along the anterior dorsal commissure, but go vertically downward through the latter. Von Alten ('10, text fig. 4A), finds a similar arrangement in the brain of *Bombus* ♀. In the workers of *Formica* and *Lasius* the ocellar nerve fibers behave like those of the *Formica* queen, going vertically downward through the posterior dorsal commissure. In the males of all three genera, however (fig. 22), the posterior dorsal commissure does serve as an "Ocellarnervenbrücke," or path for the ocellar nerve fibers, for although some fibers go vertically downward through the posterior commissure, others curve to the right or left and pass along the commissure into the protocerebral lobes. In the case of these three males, therefore, Jonescu's characterization of the posterior dorsal commissure as a "chiasmatische Bahn" is most appropriate. Jonescu states further that in the honey bee this commissure originates from cells of the intercerebral region: "Sie entsteht aus den Fasern, welche von den Ganglienzellen der Pars intercerebralis kommen." This is not the case in the ants here investigated, for in all these forms the posterior dorsal commissure can be traced into the fibrous core of the protocerebral lobes. Pietschker (p. 80), states in the text that he agrees with Viallanes, that the commissure originates from the protocerebral lobes, but in his figures 24, 29, 34, he fails to show any origin for it.

4. OCELLI, OCELLAR NERVES, OCELLAR LOBES

Ocelli are present in all three castes of the three genera in question, with the exception of the worker of *Camponotus* in which they are absent. In the workers of *Formica* and *Lasius* the ocelli are much reduced in size. The unpaired ocellus is always anterior to the paired ocelli. The nerve from the unpaired ocellus immediately divides into two branches which run backward and downward, meeting the nerves from the paired ocelli just outside the brain sheath (fig. 10).

The place and mode of entrance into the brain of these ocellar nerves varies in the different castes. The nerves enter farther forward in the queens than in the workers, and still farther back in the males. In the queens and workers the nerves from the anterior ocellus penetrate the brain sheath a short distance in front of the nerves from the posterior ocelli; in the males, all four nerves penetrate the brain at about the same point. The nerves enter the queen brain in a plane about parallel with the anterior surface of the mushroom bodies, and well in front of the central body. In the workers, the median nerves, from the unpaired ocellus, enter the brain above the anterior part of the central body, but the lateral nerves, from the unpaired ocelli, enter the brain behind the central body. The nerves of the males all enter the brain at one point, just posterior to the central body.

After their entrance into the brain the fibers from the four ocellar nerves in all three castes expand into the four ocellar lobes, fiber masses with an outer layer of nerve cells, which lie close together in the median line of the intercerebral region. The ocellar lobes of the male and queen are large, those of the worker small. The outer lobes, from the paired ocelli, are larger than the inner lobes, from the unpaired ocellus (figs. 11-13, and figs. 22-23, *i.o.l.*, *o.o.l.*).

As stated before, the fibers from the ocellar lobes reach the fibrous core of the protocerebral lobes in a different manner in the various forms of ants. In the queens of *Camponotus* and *Lasius* (fig. 14, *o.oc.l.*) the fibers go together with the posterior fibers of the anterior dorsal commissure, which lie just beneath

the ocellar lobes, some fibers crossing to the right, some to the left, and so on down into the protocerebral core. The fibers of the inner lobes enter the brain slightly anterior to those of the outer lobes, as may be seen in figure 14, *o.oc.l.*, from which the inner lobes have disappeared, but fibers from the outer lobes are curving into the anterior dorsal commissure. In the queen of *Formica* (fig. 13) there is no such relation between the anterior dorsal commissure and the ocellar fibers. All four ocellar lobes extend back to the posterior dorsal commissure, and are seen in figure 23 lying dorsal to it, their fibers running down through it into the protocerebral lobes. In this ant the fibers from the outer ocellar lobes pass down anterior to those from the inner lobes (fig. 23, *i.o.l.*, *o.o.l.*). In the workers of *Formica* and *Lasius* the four ocellar lobes blend into two masses above the posterior commissure, and their fibers run down through the commissure, interlacing and crossing from one side to the other before entering the protocerebral fibrous core. In the males of the three genera (fig. 22), the four ocellar lobes extend back to the posterior commissure; some of the fibers pass down and through it, others continue along the commissure into the protocerebral lobes.

To summarize: in ants the ocellar nerve fibers may take three different paths from the ocellar lobes into the protocerebral lobes: (1) by way of the anterior dorsal commissure, queens of *Lasius* and *Camponotus*, (2) through the posterior commissure, *Formica* queen, *Formica* and *Lasius* workers, (3) both through and by way of the posterior dorsal commissure, males of the three genera.

The second path was described by von Alten for the female of *Bombus*, and by Jonescu for the honey bee.

Berlese ('07, p. 573), states:

All'ingresso di ciascuno dei nervi ocellare si trova un piccolo ganglio (ganglio ocellare), dal quale procede il nervo ocellare. Secondo Viallanes (*Acridium*) il nervo degli ocelli laterali penetra nella parte superiore e posteriore del lobo procerebrale corrispondente. Il nervo ocellare mediano e veramente pari, perchè nel cervello si divide in due branche divergenti, di cui ciascuna va ad unirsi al nervo laterale corrispondente.

Berlese in his figure 681 shows three ocellar lobes above the posterior dorsal commissure, or "*ponte dei lobi protocerebrali*." In a note he quotes Cuccati, referring to figure 685, "*Il nervo degli ocelli pari receive fibre che partono dalle punte estreme del ponte dei lobi cerebrali ed altre che partono da cellule ganglionari della parte antero-superiore del procerebro*." It would seem that the fibers said by Cuccati to be *received* by the ocellar lobes from the apex of the "*protocerebral bridge*," the posterior commissure, were probably *leaving* the ocellar lobes by way of the commissure, as they are described in this paper in male ants.

These different accounts show that there is evidently much variation in the structure and arrangement of the ocellar lobes and the ocellar nerve fibers of insects.

5. THE OPTIC BODIES

The optic bodies, so called by Kenyon, a translation of Viallanes' term, "*tubercules optiques*," are two very small lobes on the ventral surface of the anterior part of the protocerebral lobes (fig. 10, *o.b.*). These structures are very small lobes of the fibrous core with small bundles of fibers running out from them to the surrounding nerve cells, but they are present in all three castes of the three genera here described. Pietschker ('10, p. 79) states that he does not find these bodies in *Camponotus ligniperdis*. Jonescu ('09) finds them in the honey bee, in a situation similar to that described for ants.

6. THE OPTIC LOBES

The optic lobes of the three genera under consideration consist of the three outer, middle, and inner fiber masses described in other ants and in bees. A very marked variation in the size of these lobes is observable in the different castes and in different genera, and was discussed in the section dealing with the comparison of the castes. The finer structure of the optic lobes has been so exhaustively described that it has been omitted from the present study.

7. THE CENTRAL BODY

The central body (figs. 13, 14, *c.b.*), *corps central* Viallanes, Zentralkörper, Floegel, is situated in the median line of the body, toward the posterior part of the protocerebral lobes and indeed embedded in them, for the anterior dorsal commissure arches above the central body and the ventral masses of the protocerebral lobes lie beneath it. The structure of the central body is the same in the different castes and also in the three genera under consideration, but varies in size with the size of the brain in which it is present (figs. 32-40). The dorsal part is curved in outline and consists of fibrous substance distributed into bundles which are arranged radially, and are separated by narrow spaces containing small nerve cells almost devoid of cytoplasm. A curved line of the same small nerve cells separates the dorsal from the ventral part, both of which are connected by bundles of fibers with the protocerebral lobes (figs. 13, 14).

8. THE "TUBERCLES OF THE CENTRAL BODY"

The so-called tubercles of the central body are two small fibrous masses situated beneath the posterior part of the central body, connected with it by fibers, and extending behind the central body beneath the posterior dorsal commissure, and dorsal to the protocerebral lobes (figs. 17-21). At their posterior ends these bodies enter and become a part of the fibrous core of the protocerebral lobes (figs. 21, 22).

Viallanes has termed these bodies "*tubercules du corps central*," and believes that they are associated with the central body. Kenyon considers them related to the ocellar nerve fibers, as his term "*ocellar glomeruli*" indicates. Jonescu ('09), follows the view of Kenyon in terming these structures "*Ocellarglomerulen*," but he notes that they are connected by fibers with the central body. On page 142 he states:

Die Ocellarglomerulen die man auch als Kerne der Ocellen bezeichnen könnte, zeigen auch dieselbe Form und Struktur bei allen drei Bienen formen (Drohne, Königin und Arbeitsbiene). Sie stehen in direkter Beziehung mit dem äusseren Teil des Zentralkörpers. Fasern von

der Pars intercerebralis (Haller) dringen durch dem Zentralkörper in die Ocellarglomerulen ein und gelangen dann weiter als Ocellarnervenfasern in die Ocellen. Charakteristisch finde ich einen Teil der Fasern, welche aus den inneren Kapseln der Zentralkörpers kommen und eine Kreuzung vor der Eintritt in die Ocellarglomerulen bilden (Fig. 22).

Pietschker evidently considers the "tubercles of the central body" and the "Ocellarglomeruli" as two separate pairs of structures. On page 81, under the heading "Der Zentralkörper," he states: "Der obere Teil des Zentralkörpers endigt nach hinten in zwei kreisförmigen Knoten, die links und rechts der Medianebene liegen. Sie bestehen aus derselben Substanz wie der Zentralkörper selbst, und stehen miteinander durch ein kurzes nervenbündel in Zusammenhang. Viallanes nennt diese beiden Knoten 'tubercules du corps central.'" On page 82, under the heading "Pars intercerebralis," Pietschker further states: "Die Ocellarglomerulen (Taf. 6, fig. 24 Ogl), kann man ebenfalls als Gebilde der Pars intercerebralis ansehen. Es sind dies zwei kleine Kugelförmige Gebilde aus Fasermasse, die unterhalb den protocerebralen Nervenbrücke im Gehirn liegen und mit den Ocellen in Verbindung stehen."

These structures should not be termed "tubercles of the central body," for although connected by fibers with the ventral part of the central body, they are, in my opinion, not a part of it. Neither should they be called "ocellar glomeruli," since there is no real connection between them and the ocellar nerve fibers, but merely a proximity.

These so-called 'tubercles' or 'glomeruli' are in reality continuations of the inner ends or roots of the mushroom body stalks and connect the mushroom bodies with the posterior part of the protocerebral lobes. All previous investigators of the mushroom body stalks make two statements: (1) that the anterior roots end on the anterior surface of the protocerebral lobes, (2) that the posterior or inner roots end abruptly beneath the central body. The first statement is correct, but the second is not, at least not for the three castes of the three genera of ants under consideration. In all these forms the stalks of the mushroom bodies do *not* end beneath the central body, but each stalk divides there

into two bundles (fig. 17, *st.*) so that four of these bundles, or roots, two dorsal and two ventral, are seen in sections of this region, figure 18, *d.b.*, *v.b.*, (*p.r.*). The fibers of the two dorsal bundles pass immediately upward into the central body and may be termed the *central body roots* of the mushroom bodies; the fibers of the two ventral bundles, which are identical with the so-called "tubercles of the central body," but which are actually *the posterior roots of the mushroom bodies*, continue backward and finally enter the hindermost part of the protocerebral lobes on each side of the median line of the dorsal surface (figs. 18 to 23, *p.r.*).

The identity of the posterior mushroom body roots with the "tubercles of the central body" is so clearly seen in the series of sections shown in figures 17 to 21 that it seems surprising that it has not been observed before. Jonescu has described the crossing of fibers from the central body to the "ocellar glomeruli" and indeed in his figure 22 he figures a second round body, unlabeled, on the left side of and above the 'tubercles.' This may indicate that, in the honey bee, the ventral rather than the dorsal bundles, or roots, are connected with the central body, but it is, in my opinion, strong evidence that the "ocellar glomeruli" are identical with the posterior roots of the mushroom bodies in the honey bee as well as in ants.

9. THE MUSHROOM OR PEDUNCULATE BODIES

The mushroom bodies have always been objects of great interest, both on account of their structural prominence and of the various functions assigned to them by different writers. They have been termed by Dujardin ('50) "*les corps pédunculés*;" by Leydig, "*die gestielten Körper*;" "mushroom bodies," by Kenyon; "pedunculate bodies," by Wheeler; "*corpo peduncolato*," Berlese.

The finer structure of the mushroom bodies may be seen best in frontal sections of the brain. Figures 32 to 40 represent diagrams of the mushroom bodies, with their fiber tracts and cell groups, as if seen in optical section. The outlines of these figures

were drawn with the camera lucida from sections through the central part of each mushroom body, and the fiber tracts reconstructed from other sections. Figures 27 and 29 show the cells in detail.

Each mushroom body (figs. 12, 13) consists of two parts, an inner lobe, and an outer lobe. The inner lobe projects slightly farther forward and is a little wider from side to side than the outer lobe, but in an antero-posterior direction, the two lobes have the same measurement. Each lobe consists of an outer layer of nerve cells and an inner fibrous portion, deeply indented at its distal end, forming the cup or calyx, and continuing downward as the stalk. The inner and outer stalks of each lobe unite to form one common, main stalk (fig. 12, *st.*), which penetrates deep into the fibrous core of each protocerebral lobe. The fibers coming from the two lobes remain distinct throughout the greater part of the stalk.

a. The roots of the mushroom bodies

The anterior roots of the mushroom bodies arise from the main stalks just below the junction of the stalks from the inner and outer lobes, in the region that is known as the 'crossing' or 'decussation' of the fibers (fig. 26).

Careful examination of the sections in this region proves that there is no crossing or decussation in the sense of the passing of fibers from one side of the stalk to the other, but that there is a gradual bending out of three or four very small bundles of fibers from the longitudinal plane, that of the main stalk, into the transverse plane. These four fiber bundles may be traced forward and constitute the anterior roots of the mushroom bodies. Figure 24 shows the anterior root with four bundles of fibers, two derived from the outer, two from the inner lobe. Some fibers are seen passing between the root and the protocerebral tissue. In figure 25, some distance anterior to the last figure, the two bundles of each side have fused into one. An interchange of fibers between the root and the protocerebral core is still taking place and continues throughout the remaining length of the root. It is impossible to state whether the direction of the fibers is in-

ward or outward, but the fact that the diameter of the root is slightly greater at its anterior end seems to indicate that some fibers must enter as well as leave the stalk. Anterior to figure 25 the two bundles of transverse fibers fuse into one (fig. 10). The anterior root ends on the antero-dorsal surface of the protocerebral core in a loose network of fibers.

The posterior roots of the mushroom bodies have already been described under the heading "Tubercles of the central body." To restate briefly: The distal end of each stalk, the previously so-called 'inner' root, divides beneath the central body into two bundles, a dorsal and a ventral, derived evidently from the fibers from the inner and outer lobes; the two dorsal bundles pass into the central body as the central body roots of the mushroom bodies, the two ventral bundles, or the posterior roots of the mushroom bodies, continue backward and enter the dorsal surface of the hindermost part of the protocerebral lobes, in the region where the protocerebral lobes are connected with the subesophageal ganglion.

b. The nerve cells of the mushroom bodies

The cells of the mushroom bodies as seen in frontal sections (figs. 32-34) are distributed into groups or zones which are usually separated from one another by narrow spaces, so that the outlines of the groups may be distinguished even with a low power. The study of these cell groups with an oil immersion lens reveals slight but constant differences in the size and arrangement of the cells (fig. 29). The groups are the same in both outer and inner lobes. Four kinds of cells are distinguishable; in an entire lobe these are arranged in four zones encircling the calyx, but in sections they appear as seven cell groups, described here for the first time. In figure 29, Group I, is the oval mass of large cells in the center of the calyx or cup; Groups II, *l.*, II, *r.*, are the broad fan-shaped masses of cells occupying most of the dorsal surface of the lobe; Groups III, *l.*, III, *r.*, are broad aggregations of cells forming the sides of the cup; Groups IV, *l.*, IV, *r.*, are small masses of cells at the base of the cup. Only the nuclei of these cells are drawn in figure 29, but cell bodies are shown in

figure 27. It will be noted that the amount of cytoplasm is very small in all the mushroom body cells.

Group I (figs. 27, 29) forms a prominent oval mass of large cells which are much larger than those of any other group. Both cell body and nucleus are large, the nucleus is oval, with a large nucleolus. The axons of these cells unite in a conspicuous bundle, *h*, that runs downward in the center of the stalk (figs. 32 to 40). These efferent fibers give reason for regarding this cell group as probably an important motor center.

The Groups II are shaped like a fan or broad wedge, being arranged in radiating rows of ten or twelve cells whose fibers converge into definite and prominent bundles, *g*, also efferent in nature. The two bundles *gl.* and *gr.* from each Group II pass into the center of the stalk together with, but on the outside of, the bundle *h*, from Group I. The cells of Group II (fig. 27), are slightly elongated with oval nuclei and very little cytoplasm.

Group III is usually distinctly separated from Group II by a narrow space, or its outline may be determined merely by the different size and arrangement of the cells. The surface toward Group III may likewise be bounded by a slight space from which cells are absent, or the two groups may merge into one without any line of separation. The cells of Group III are very little smaller than those of Group II, but it will be noted in figure 27 that there is less cytoplasm, that the cell bodies are not elongated and that the nucleus is rounder. These cells are usually arranged in vertical rows or layers, seven or eight cells deep. The fibers from these cells run into the apices of the calyx, but the bundles can rarely be traced very far. Tracts *e*, *r*, *m*, *n*, to be described below, originate from the cells of Group III.

Group IV forms the basal part of the cell envelope, and is the smallest group, both in the size and in the number of the cells. The cytoplasm forms merely a narrow rim around the small round nucleus.

These four zones of cells, seen in section as seven groups, are present and typical in all three castes of the three genera under discussion. Figure 27, from the worker of *Lasius*, and figure 29, from the worker of *Camponotus*, show that the largest cells of

the cup, Group I, are present in the worker as well as in the queen and male. On this point I differ from Pietschker, who does not find these large cells in the worker of *C. ligniperdis*, but only in the sexual forms. Pietschker ('10, p. 77): "Bei den Geschlechtsformen fand ich die Höhlung der beiden Becher von einer Masse auffallend grosser Zellen angefüllt (Taf. 5, fig. 5, 6 z), wie ich sie bei der Arbeiterin nicht zu bemerken vermochte. Die letztere zeigt kleinere Zellen, welche um so zahlreicher sind."

Pietschker also fails to note the arrangement and differentiation of the mushroom body cells into the groups described above.

Berlese ('09) refers to the small size of the mushroom body cells and to the reduction in their cytoplasm, but does not mention the greater size of the cells in the cup, Group I. On page 574 Berlese states:

La cavita del calice e riempita di cellule cromatiche molto piccole (fig. 669 Cr), con protoplasma assai ridotto. La sue parete e formata di sostanza punteggiata, a trama molto serrata. La cellule inviano i loro prolungamenti a questa parete: il fusto trae le fibre che lo compongono, non direttamente dalle cellule ma dalla parete del calice. Questa parete si unisce, all in dentro, alla sostanza del lobo procerebrale a mezzo di un tratto fibroso. Il fusto apparisce cosi formato da un fascio di fibre parallele.

Kenyon states that "the cells of the hexapods generally . . . are strongly distinguished from nerve cells elsewhere in the brain, and in the whole nervous system for that matter, in being nearly devoid of extra nuclear protoplasm. This fact led Dietl to term them ganglionic nuclei." Kenyon found that "The cells filling the cup appear to be of two kinds . . . Laterally they are much larger than those in the middle."

Jonescu, in the mushroom bodies of the honey bee, describes three groups of nerve cells filling the cavity of the cup, seen in section as a median and two lateral groups, although in reality the lateral groups form a circle about the median group. The cells of the median group are larger than the lateral ones. Jonescu followed the fibers from these cells into the stalk, and found that the fibers from the large median cells occupied the very center of the stalk with those from the lateral cells outside. The remaining cells of the mushroom body are described as lying

on the outside of the cup and are termed by Jonescu the "ganglion cells of the wall."

The conditions described by Jonescu in the honey bee bear a very close resemblance to those of ants. The three cell groups of the bee, the median and two lateral, situated in the cup, are evidently homologous with the Groups I, II, *l.*, II, *r.*, of ants. The wide calyx of the bee contains three cell groups within it, but from the narrower cup of the ant the cell groups II, *l.*, and II, *r.*, have been crowded upward and outward. The fibers, however, are completely homologous in the two insects; the central fibers of the stalk, from the median cell group, in the bee correspond to the fiber tract *h* in ants, and the more lateral fibers of the stalk, from the lateral cell groups, in the bee correspond to the tracts *g* of ants. It should be noted that Jonescu has found the large median cells in the worker, for his figure 36 is from the brain of a worker bee. It would be interesting to know whether the slight but constant differences observed between the cells of the Groups III and IV in ants may not also exist in the "ganglion cells of the wall" of the honey bee.

c. Fiber tracts of the mushroom bodies

Definite fiber tracts arise from the cell groups and can be traced into the stalks either of the same or of the adjacent lobe, or out of the stalks into the fibrous core of the protocerebral lobes. The origin of some of these fiber tracts in the cell groups can be seen with such distinctness that their efferent nature may be assumed; but although afferent tracts are doubtless present I have not been able to distinguish them as such.

The tracts observed are the same in both right and left mushroom bodies, but differ in the outer and inner lobes of a single mushroom body. The detailed descriptions which follow show that many tracts are present throughout the different castes and genera, while others are confined to one caste, and still others are variable. The tracts that are of constant occurrence in all the castes of the three genera here described are: *b*, *fl*, *h*, *gl*, *gr*. *B* is really a protocerebral fiber tract, uniting the outer and inner protocerebral regions, but is connected by fibers with the

mushroom bodies. The fibers of *fl* unite the inner and outer lobes. *H*, *gl.*, and *gr.* are efferent fiber tracts occupying the core of the stalk, and originating respectively from the cells of Groups I and II.

More tracts are found in the brain of the queen than in the worker or male. This is not due to size, since the worker mushroom bodies are larger than those of the queen in *Camponotus* and *Formica*, and about equal in *Lasius*; it therefore seems to indicate that the queen possesses the most complex and highest type of brain. The tracts that are characteristic of the queen are tracts *c*, *cx*, and *d*; *c*, however, being present in one male, *Camponotus*. *R* is found only in the queens of *Formica* and *Lasius*. *E* is absent from the queen brains of *Formica* and *Lasius*, but is present in the *Camponotus* queen. It is interesting to note from table 2 and from the figures that the brain of the queen of *Lasius niger*, which has already been spoken of as possessing a more generalized type of structure than *Camponotus* or *Formica*, has a few more tracts than either. But the same table and figures make it evident that the decreased size of the mushroom bodies of any caste is due to a decrease in quantity, common to all cells and fibers, rather than to the disappearance of particular cell groups or fiber tracts.

Camponotus queen: figure 32. The study of the mushroom bodies by means of serial sections cut in the frontal plane shows the following fiber tracts named from the anterior surface backward. Tract *b* lies anterior to the main stalk of the mushroom body, connecting the outer and inner parts of the protocerebral lobes, and on the dorsal surface is connected with other fibers of the mushroom body, although these fibers are not shown in the figure. This tract, which is three sections thick in its central part, is a broad very prominent bundle of fibers, curving from the outer part of the protocerebral lobe up as far as the base of the cellular envelope of the mushroom body, then down again into the inner part of the protocerebral tissue on one side of and dorsal to the central body. In the queen of *Camponotus* there is nothing to indicate the direction of the fibers, but in the workers of *Camponotus* and *Formica* (figs. 33 and 36), the origin of *b* can be traced

TABLE 2
Showing fiber tracts of the mushroom body

ORIGIN AND TERMINATION OF THE FIBER TRACTS	CAMPONOTUS						FORMICA						LASIUS			
	Queen		Worker		Male		Queen		Worker		Male		Queen		Worker	
	outer lobe	inner lobe	outer lobe	inner lobe	outer lobe	inner lobe	outer lobe	inner lobe	outer lobe	inner lobe	outer lobe	inner lobe	outer lobe	inner lobe	outer lobe	inner lobe
Cells of mandibular lobe, up to outer part of protocerebral lobe, in front of mushroom body stalks to the inner part of protocerebral lobe. <i>C</i> from Group III _r , inner lobe to outer part of protocerebral lobe. <i>Cz</i> from Group III <i>L</i> , outer lobe.....	b		b	b	b	b	b	b	b	b	b	b	b	b	b	b
Group IV <i>L</i> , inner lobe, to inner part of protocerebral lobe.....	c			c			c			c			cx			
Group III <i>L</i> , inner lobe, to inner part of protocerebral lobe.....	d			d			d			d						
between Groups IV of the two lobes. <i>β</i> between Groups III and IV of the two lobes.....	e			e					e						e	
Groups II <i>r</i> , of each lobe to stalk.....	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f
Groups I of each lobe to stalk.....	gl-gr.	gl-gr.	gl-gr.	gl-gr.	gl-gr.	gl-gr.	gl-gr.	gl-gr.	gl-gr.	gl-gr.	gl-gr.	gl-gr.	gl-gr.	gl-gr.	gl-gr.	gl-gr.
Connecting Groups III of the two lobes.....	h	h	h	h	h	h	h	h	h	h	h	h	h	h	h	h
Group III <i>r</i> , outer lobe, to stalk.....	l	l							l							
Group III <i>r</i> , outer lobe, to outer part of protocerebral lobe.....	m		m				m		m		m		m		m	
Groups III <i>r</i> and IV <i>r</i> outer lobe, to outer part of protocerebral lobe.....					p		p		p		p		p		p	
Group III <i>L</i> , inner lobe, to stalk.....							r						r			
Total number of fiber tracts.....	10		9		9		11		9		9		12		7	

to cells in the mandibular lobe. There is an opportunity for an exchange of fibers between *b* and the mushroom body, since the fibers of each touch one another without any intervening sheath, and in places fibers may be seen passing out of the cup into *b*.

Tract *c* consists of fibers arising in Group III, *r*. of the inner lobe. The fibers run downward and outward, crossing in front of the stalk of the outer lobe, dorsal to the lateral part of *b*, and entering the protocerebral tissue just lateral to and in close contact with the fibers of *b*. The central part of *c* is present in three sections. This is a very noticeable tract on account of its direction and its size. It is present in all three queens and in the male of *Camponotus*.

Tract *d*, present only in two sections, is a narrow inconspicuous bundle of fibers arising from Group IV, *l*, of the inner lobe and ending in the inner protocerebral tissue. Its fibers sometimes pass through the anterior protocerebral commissure. *D* is a tract that has been found only in the queen caste.

Tract *e*, present in two sections, originates in Group III, *l*, of the inner lobe. Its fibers occupy most of the inner part of the calyx, then run downward and inward, and leaving the stalk of the inner lobe, terminate in the protocerebral tissue posterior to and on the median side of the terminal fibers of *d*. *E* is found in all castes of *Camponotus*, but only in the workers and males of *Formica* and *Lasius*.

Tract *f* is present in two sections and is a narrow band of fibers connecting the outer and inner lobes. The fibers seem to arise from the Groups IV of both lobes, but it is impossible to state whether the fibers run in both directions or only in one.

Tract *l*, a curved band of fibers lying beneath *f* and separated from it by a space filled with supporting cells, lies two sections posterior to *f* and is quite distinct from it in the queen of *Camponotus*. In all the other forms *f* and *l* are united into either a simple or a double commissure, and are present therefore in the same sections. *L*, as seen in the *Camponotus* queen, is a rather stout band of fibers running between the outer and inner lobes, with its central part at a more ventral level than *f*, but arising at a

higher level, probably from the cells of Group III. It is impossible to state whether the fibers all run in the same or some in the opposite direction from side to side.

The paired tracts *g* are present in both lobes, originating in the cells of both Groups II and forming the main bulk of each stalk in the two parallel bundles *gl.* and *gr.* Tract *h* arises in both lobes from the cells of Group I. Its fibers form a fan-shaped mass, rapidly converging into a slender bundle which occupies the center of the stalk, bounded on the outside by the paired bundles of *gl.* and *gr.* Tract *m* originates in Group III, *r.* of the outer lobe. Its fibers run downward on the outer surface of the stalk.

Camponotus worker: figure 33. The first tract to be found in the worker is *b*, homologous with that of the queen, but in this caste its origin can be determined. Tract *b* originates in the large nerve cells of the cortex of the mandibular lobe. Bundles of fibers can be seen leaving these cells, entering the fibrous core of the mandibular lobe, passing upward into the outer part of the protocerebral lobe and thence into the bundle which like that of the queen curves in front of the outer and inner stalks of the mushroom body, in contact with its fibers, and finally passes toward the median line in a curve. Then downward, on its way passing through the anterior protocerebral commissure, and going deep into the protocerebral tissue near the central body. Tracts *c* and *d* are absent in this worker, tract *e* is similar to that of the queen. Tract *fl*, which probably represents both *f* and *l* of the queen, is a single commissure uniting the inner and outer lobes. The dorsal part, apparently arising from the two Groups IV, is slightly denser than the loosely arranged ventral part, which arises from a higher level. At both ends the fibers may be seen to arise from the cells of Groups III and IV. It is therefore evident that some fibers must run in each direction in this tract. Tract *h* and the paired tracts *gl.* and *gr.* are similar to those of the queen. Tract *n*, present in two sections, is a very slender but distinct fiber bundle. It originates in Group III, *r* of the outer lobe, and leaving the stalk, terminates in the outer part of the protocerebral tissue. *N* may

be the same as *p* of other forms, that is, some of its fibers may come from Group IV as well as from III, but since no connection with Group IV could be seen, it has been classed as a separate fiber tract and is separately lettered.

Camponotus male: figure 34. Tract *b* is homologous with that of the queen. Its origin cannot be traced to the mandibular lobe as in the case of the worker. Tract *c* is similar to that of the queen. Tract *d* is absent. Tract *fl* is similar to that of the worker. Tracts *e*, *h*, *gl.*, *gr.* are homologous with those of both queen and worker. Tracts *m* and *n* are absent. Tract *p* is a slender fiber bundle which arises from both Groups III, *r* and IV, *r* of the outer lobe, and leaving the stalk, enters the outer part of the protocerebral lobes.

Formica queen: figure 35. Tract *b* is well developed and is homologous with *b* of the other forms. Its origin cannot be traced beyond the protocerebral tissue. Tract *c* has a slighter development in *Formica* than in either of the other queens. It exists merely as a few delicate strands coming from the inner cup rim of the inner lobe and entering the protocerebral tissue together with the outer limb of *b*. Tract *d* is homologous with *d* of the other queens, *fl* is homologous with that of the *Camponotus* worker and male, *e* is absent from this caste. Tracts *h*, *gl.*, *gr.*, are similar to those of all the other castes and genera. Tract *m*, though present is very slender, tract *p* is similar to *p* of other forms. A new tract, *r*, is seen for the first time in this form. It arises from Group III, *l*, of the inner lobe and runs down the outer surface of the stalk of this lobe.

Formica worker: figure 36. The origin of *b* from a stout bundle of fibers that is traceable to cells in the cortex of the mandibular lobe is more distinct in the *Formica* worker than in any other form. Tracts *c* and *d* are absent, *e* is slender and delicate but distinct. *F* and *l* are two fiber tracts which are present in the same three sections, and unite the inner and outer lobes, *f* dorsal to *l*. With the exception of the fact that these two tracts occur one above another in the same sections they are exactly similar to *f* and *l* of the *Camponotus* queen. Tracts *h*, *gl.*, *gr.*, *m*, and *p* are similar to those of other forms.

Formica male: figure 37. Tract *b* is similar to that of the queen. Tracts *c* and *d* are absent. Tract *fl* is similar to that of the queen. Tract *e* is similar to that of other forms. Tracts *h*, *gl.*, *gr.*, are homologous with those of all other forms. Tracts *m* and *p* are similar to those of other forms.

Lasius queen: figure 38. Tract *b* is homologous with *b* of other forms, except that its origin cannot be traced beyond the protocerebral lobes, but the connection of *b* with fibers from all parts of the mushroom bodies is especially well seen in this form. Tract *c* is very prominent, and in addition to this is a new tract *cx*, consisting of fibers from Group III, *l.* of the outer lobe. *Cx* has been found only in the *Lasius* queen. Tract *d* is homologous with *d* of other queens, *e* is absent. Tract *fl* is a single commissure homologous with *fl* of other forms. Tracts *h*, *gl.*, *gr.*, *m*, *p*, are homologous with other forms. Tract *r*, from Group III, *l.* of the inner lobe, is similar to tract *r* of the *Formica* queen, the only other form in which this tract has been found.

Lasius worker: figure 39. Tract *b* is homologous with other forms in which the origin cannot be traced beyond the protocerebral lobes. Tracts *c* and *d* are absent. Tract *e* is slender but typical. Tracts *fl*, *h*, *gl.*, *gr.*, *m*, are similar to those of other forms.

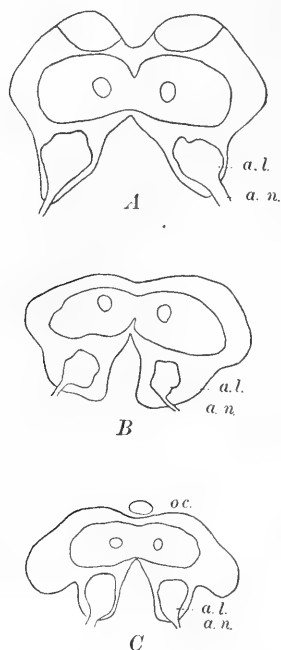
Lasius male: figure 40. Tract *b* is similar to all other forms in which the origin is traceable only to the protocerebral lobes. Tracts *c* and *d* are absent. Tracts *e*, *fl*, *h*, *gl.*, *gr.*, *n*, *p*, are similar to those of other forms.

10. THE ANTENNARY LOBES, OLFACTORY LOBES

The antennary or olfactory lobes, the 'deutocerebrum' of Viallanes, are paired lobes situated in the anterior and ventral surface of the brain and connected on the dorsal surface with the protocerebral lobes. In the three castes of ants the antennary lobes are relatively about the same size, in proportion to the size of the brain, being smaller in the always smaller male brain, or in a small queen brain, for example, *Formica*. Judging by external size, see figures 1 to 9, the antennary lobes of the castes of the

three genera under consideration may be graded as follows: *Camponotus*, figures 1 to 3, queen, worker, male; *Lasius*, figures 7 to 9, queen, worker, male; *Formica*, figures 4 to 6, worker, queen, male.

The sections drawn in text figure 2, from *Camponotus*, all taken through the point of exit of the antennary nerves, show that at this point the entire antennary lobes of the queen and worker are about equal, although the fibrous core in the queen



Text fig. 2 Sections of the brain of *Camponotus pennsylvanicus* through the antennary lobes at the point of exit of the antennary nerves. *A*, queen, *B*, worker, *C*, male. *a.l.*, antennary lobes; *a.n.*, antennary nerves; *oc.*, ocellus.

A, is much larger than in the worker, *B*. Posterior to this region the fibrous core of the queen decreases in size and the cell envelope increases, but the fibrous core of the worker is never larger than in this figure.

Pietschker finds these lobes larger in the worker than in the queen. On page 88 he states: "Der Lobus olfactorius besitzt

bei den 3 Formen eine verschiedene Grösse. Bei der Arbeiterin ist er verhältnissmässig sehr gross entwickelt, beim Männchen am kleinsten, während das Weibchen ungefähr in der Mitte zwischen beiden steht. Eine Vergleichung der Tafelfiguren 21 bis 33 (L.a.) führt uns dies schon äusserlich vor Augen." Pietschker's text figure 14 also shows that the fibrous core of the worker is larger than that of the queen.

The origin and distribution of the antennary nerves has been studied only in the queen of *Camponotus*, but my observations in this form are entirely in accord with those of Janet ('05), in the brain of *Lasius niger*. The five pairs of antennary nerves of the *Camponotus* queen leave the antennary lobes in two bundles, the first anterior, and arising from the outer, lateral surface, the second posterior, and arising from the inner or median surface. From the anterior bundle arise three nerve trunks, two large and one small, and a second small nerve arises from one of the large trunks. From the posterior bundle a single trunk originates, dividing almost immediately into four branches. These nerves correspond to the five pairs of nerves described by Janet, and are named as follows: from the anterior bundle (1), (2), two large nerves which are the "antennary sensory nerves I and II" of Janet; (3) the small nerve trunk is the antennary motor nerve to the segments of the antenna, the "funicular nerve" of Janet; (4) the second small nerve arising from one of the larger trunks is the "sensory chordotonal nerve" of Janet; (5) the nerve from the posterior bundle, which divides into four branches, is the "motor nerve" of Janet, supplying the four muscles of the basal segment or scape of the antenna.

11. THE TRITOCEREBRAL LOBES, AND THE TRITOCEREBRAL NERVE

The tritocerebrum, or the region corresponding to the third head segment, is very much reduced in the ant. The tritocerebral nerve (fig. 16, *tr.n.*), which is median and unpaired and lies beneath the esophagus supplying the inferior dilator muscle of the pharynx, has paired roots which arise from two very small

fibrous masses and a few ganglion cells situated on the inner surfaces of the subesophageal ganglion. The fibrous masses (fig. 16, *tr.l.*), from which the roots arise, project so slightly that they hardly deserve the name of lobes, and yet they represent all that remains of the tritocerebrum. This region is homologous in all castes of the three genera of ants described in this paper.

It has been stated already that Janet ('05), found and described the small nerve which supplies the inferior dilator muscle of the pharynx in *Lasius*, which he considered to be the tritocerebral nerve, although he did not trace it to its origin.

Jonescu ('09), found a similar nerve in the honey bee and succeeded in tracing its origin to two small projections from the subesophageal ganglion which he therefore termed the tritocerebral lobes. Pietschker ('11, figs. 1-3), shows that the tritocerebral nerve of *C. ligniperdis* arises in the same way and in the same position as in the honey bee.

12. THE SUBESOPHAGEAL GANGLION

The subesophageal ganglion is united with the protocerebral lobes by the dorso-ventral connectives (fig. 15). This ganglion represents the last three of the six head segments now fused into one mass. From its ventral surface arise the paired nerves of the mouth parts, the very stout mandibular nerves, the maxillary nerves, the labial nerves; from the dorsal surface arise the paired salivary nerves. I have not yet found the paired accessory nerves described by Pietschker, but, as I have given very little attention to this part of the brain, I do not feel prepared to state definitely that they are absent from my preparations. At its posterior end the esophageal ganglion narrows into the ventral nerve cord.

SUMMARY

I. STRUCTURE

1. *The mushroom bodies*

a. Cells. Four kinds of cells, the cells of Groups I, II, III, IV, are present in the mushroom bodies arranged in four zones, but seen in section in seven groups. These cells differ in size and arrangement. Group I, in the center of the cup, consists of the largest cells; Group II, forming the apex of each lobe, consists of smaller cells in radial rows; Groups III and IV form the sides of the cup and are composed respectively of small and very small cells. Group I is found in worker ants as well as in queens and males.

b. Fiber tracts. The fibers from Groups I, II, and some from III, can be traced into the stalks and form the chief efferent fiber tracts of the mushroom bodies.

The fibers are combined into bundles or tracts that have a definite and constant arrangement. Some tracts are present in all castes of the three genera, some are confined to the queen caste, and others vary in occurrence. The queen brain has more fiber tracts than the other castes, and the queen of *Lasius* more than either *Formica* or *Camponotus*.

c. Roots. The anterior roots of the mushroom bodies end as described by other writers: on the anterior surface of the protocerebral lobes. The stalks or 'inner roots' of the mushroom bodies do *not* end abruptly beneath the central body as heretofore described, but each stalk divides there into two bundles or roots, making two dorsal and two ventral masses. The fibers of the two dorsal bundles enter the central body, and may be termed the *central body roots*, the two ventral bundles, which are the *posterior roots of the mushroom bodies*, continue backward and enter the posterior part of the protocerebral lobes on their dorsal surface. These posterior roots of the mushroom bodies are identical with the structures described by other writers as the "tubercles of the central body" and the "ocellar glomeruli," neither of which terms can be correctly applied to the three ant genera in question.

2. The dorsal protocerebral commissures

The dorsal surfaces of the lateral parts of the protocerebral lobes are connected by two bands of fibers termed respectively in this paper, the anterior dorsal, and the posterior dorsal commissure. The first of these is probably of compound nature. Both commissures may serve as paths for the ocellar nerve fibers.

3. The ocellar lobes and nerves

The ocellar nerves enter the brain farther forward in the queen than in the worker or male. The nerve from the anterior ocellus divides into two, those from the posterior ocelli remain single; within the brain these four branches enlarge to form the four ocellar lobes which usually remain separate, but sometimes fuse into two masses. The fibers from the ocellar lobes pass down into the protocerebral lobes by different routes in the different castes and genera.

There is no connection between the ocellar nerve fibers and the so-called "ocellar glomeruli," which are really the posterior roots of the mushroom bodies.

4. The central body

The central body consists chiefly of fibrous tissue. It is connected by fibers with the protocerebral lobes and with the mushroom body stalks.

II. COMPARISON OF THE CASTES

In the three genera under discussion the queen has the largest head and brain. The brain appears laterally extended on account of the large laterally placed optic lobes. The mushroom bodies are either equal to those of the worker, *Lasius*, or are smaller than those of the worker, *Camponotus*, *Formica*. The antennary lobes are larger than in the worker, *Camponotus*, *Lasius*, or smaller, *Formica*.

The worker brain has usually greatly reduced optic lobes and eyes. In size the mushroom bodies exceed or equal those of the queen.

The male has the smallest head and brain, with usually the largest eyes. The optic lobes are very large and curve downward. The mushroom bodies, though actually smaller than in the other castes, are relatively large in proportion to the size of the brain. The same is true of the antennary lobes.

The queen of *Lasius niger* has a more generalized and superior type of brain than the queens of *Formica* and *Camponotus*, which show evidence of reduction or degeneration, especially in the mushroom bodies.

The queen brain in its typical generalized condition, as for example in *Lasius niger*, is superior to and more highly developed in all respects than that of the worker; therefore, the queen brain represents the primitive generalized type from which the worker has been derived.

DISCUSSION OF RESULTS

The great complexity and the high degree of differentiation revealed by a close study of the mushroom bodies are additional evidence in favor of the view held by many investigators of the ant brain—notably Dujardin, Leydig, Forel, Viallanes—that these bodies are the chief motor and psychic centers of the brain. The differentiation of the mushroom body cells into groups, the number, constancy, and definite arrangement of the fiber tracts arising from these cells or ending about them, and the connection of the mushroom bodies with the other parts of the brain are points of structure that indicate their importance. If the Cajal method, tried so successfully by Jonescu for the honey bee, can be made to succeed with ants, as I hope later to do, I am convinced that even more complex arrangements of fiber tracts will be revealed.

The discovery of the connection of the posterior roots of the mushroom bodies with the protocerebral lobes should fill a large gap in our knowledge of these bodies. If the mushroom bodies are the important motor and psychic centers of the brain it seems strange that their connections with other parts of the brain should be as slight as has been heretofore described. The previously described connections of the mushroom bodies with

other parts of the brain are as follows: connected by three sets of fibers with the optic lobes, Kenyon, connected by fibers with the protocerebrum, Bellonci, Viallanes, while all writers are agreed that the main bulk of the fibers pass into the stalks, thence in smaller part to the anterior roots ending on the anterior surface of the protocerebral lobes, in larger part to the 'inner roots' which were said to end abruptly beneath the central body without visible connection with other parts of the brain.

I have shown that the anterior dorsal protocerebral commissure and the median protocerebral tissue are connected with fibers from the mushroom body tracts, *d*, *b*, *e*, and probably others; that the tracts, *c*, *p*, *n*, connect with the lateral protocerebral tissue, but that the largest, most prominent fiber tracts, *h*, *gl.*, *gr.*, originating in the largest cells, those of Groups I and II, and certainly efferent in nature, together with the smaller tracts *m*, and *r*, from Group III, form the main bulk of the stalks, and that the greater part of their fibers can be traced into the so-called 'inner roots,' which are said to end beneath the central body, but which, however, do not so end, but are distributed in part to the central body, in part, as the posterior roots of the mushroom bodies, to the protocerebral lobes at the point where these are connected with the subesophageal ganglion and consequently with the ventral nerve cord. Granted that the fibers of the stalk are efferent, motor, fibers, the question at once arises, what part could they play by merely running into the 'inner roots' and ending there? Is it reasonable that the chief efferent fibers of the body should end abruptly leaving no trace behind and without connection with other nerve centers? With the desire to trace some connection with the inner ends of the mushroom body stalks I have studied this region with great care and have returned to the problem again and again, until I am now confident that the following description is correct. The great efferent tracts from the mushroom bodies which may be traced into the stalks, and which go in small part to the anterior roots, do not terminate beneath the central body, but there each stalk divides into two roots. One root, the dorsal bundle, goes to the central body and through the central body connects by fibers with the proto-

cerebral lobes and may represent the efferent fibers or the motor system of the head; the other root, ventral in position, the posterior root of the mushroom body, continuing backward to the protocerebral lobes, subesophageal ganglion, and ventral nerve cord, may represent the efferent, motor system for the body. To carry the comparison frequently made with the vertebrate brain a step farther, if the mushroom bodies can be compared with the cerebrum, the posterior root fibers may be likened to the pyramidal tracts of the spinal cord. This explanation is founded on facts to be observed in any series of sections of the ant brains described in this paper, and it also supplies, in my opinion, the missing factor in the entire continuity of the nervous system of the ant.

Any conception of the special function of the central body seems far from clear. Here is a small highly differentiated organ, constant in structure, in position, and in its occurrence in all castes and genera of ants. It is connected by fibers with the ventral part of the protocerebral lobes and with the roots of the mushroom bodies. By these connections the central body must be in direct communication with the chief centers of the brain, but the question of its functional relation to these centers remains as yet unanswered.

COMPARISON OF THE BRAINS OF THE CASTES

In comparing the brains of the different castes and genera of ants, the question arises: Which is the generalized primitive type? Most writers are agreed that the worker brain is more highly developed than that of the queen. Wheeler, on the contrary, dissents from this view on the ground that many queens are as highly developed as the workers. On page 55, speaking of the view of Forel that the mushroom bodies are larger in the worker than in the queen, Wheeler refers to a series of *Formica glacialis* (fig. 29), showing that:

The pedunculate bodies (p. 6) are as highly developed in the female as they are in the worker, and they can hardly be said to be vestigial in the male. In *Pheidole instabilis* (fig. 30), too, the female and soldier have well developed pedunculate bodies, though these seem to be insigni-

nificant in the male. While, therefore, the male brain in all these species, apart from the huge development of its optic ganglia and stemmatal nerves, is manifestly deficient, I doubt whether we are justified in regarding the brain of the female as being inferior to that of the worker. It is true that the worker brain is relatively larger, notwithstanding the smaller eyes and stemmata, or the complete absence of the latter, but I would interpret this greater volume as an embryonic character. The worker is, in a sense, an arrested, neotenic or more immature form of the female. . . .

The queen brain seems to me to represent the generalized type from which the worker caste has departed, and, while some queens are notably degenerate in brain structure, others have remained in a far more generalized condition, for example, the queen of *Lasius niger*. If we select as a standard the degree of development of (1) the optic apparatus, including the eyes and optic lobes, (2) the mushroom bodies; and compare the castes of the three genera under consideration with the queen of *Lasius niger*, we shall obtain evidence of divergence from this type in the two opposite directions of increase, and of reduction of parts, or degeneration. From this comparative study (figs. 1-9) two facts are worthy of note: (1) the *Lasius* queen has a more highly developed, generalized type of brain than either of the queens of *Camponotus* or *Formica*, (2) the queen brain in its most highly developed, typical condition, as in *Lasius niger*, is superior to and more highly developed than that of the worker. Therefore, the conclusion is justified that the queen brain is the primitive type from which by degeneration and specialization of structure the worker brain has been derived.

If the cause of the reduction or degeneration noted in the brains of the queens of *Camponotus pennsylvanicus* and *Formica schaufussi* is sought, may it not be found in the habits of these queens? In a footnote to page 57, Wheeler ('10) states that the queen of *Lasius fuliginosus*, described by Forel as possessing a brain inferior to that of the worker, is but little larger than the worker, and is probably a temporary parasite. This is evidently a case where habit and structure are related. The male brain is another example of the inter-relation of habit and structure, since the great development of the compound eyes and optic lobes may be attributed to the need to discern and follow the

female. The cause of the decrease in the size of the mushroom bodies in the queens of *Camponotus* and *Formica* is not so clear. If the mushroom bodies are the chief motor centers of the brain, their diminution may mean a lessened amount of motor activity. It would be interesting to have minute and detailed information in regard to the habits of these different queens, such as Buckingham ('11) has given for worker ants. Is *Camponotus* perhaps less active than *Lasius*? Does the period of flight end sooner in one than in the other? Are the wings retained for a longer or shorter time?

It has been suggested by Professor Wheeler that I should study the brain of the queen of *Formica consocians*, which is parasitic in habit and also reduced in size. *Formica exsectoides*, whose queen is likewise parasitic but not smaller, may also prove of interest. These forms and others, such as the *Eciton* workers with degenerate compound eyes, will form the basis of a later paper.

May 31, 1913

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EXPLANATION OF PLATES

All figures are drawn with the Zeiss camera lucida.

ABBREVIATIONS

<i>a.cm.</i> , anterior dorsal commissure	<i>p.cm.</i> , posterior commissure
<i>a.l.</i> , antennary lobe	<i>p.l.</i> , protocerebral lobe
<i>a.r.</i> , anterior root	<i>p.r.</i> , posterior root
<i>c.b.</i> , central body	<i>r.n.</i> , recurrent nerve
<i>d.b.</i> , dorsal bundle	<i>sb.g.</i> , subesophageal ganglion
<i>i.f.</i> , inner fiber mass	<i>st.</i> , stalk
<i>i.o.l.</i> , } inner ocellar lobes	<i>tr.l.</i> , tritocerebral lobe
<i>i.oc.l.</i> , }	<i>tr.n.</i> , tritocerebral nerve
<i>lb.n.</i> , labial nerve	<i>v.b. (p.r.)</i> , ventral bundle (posterior root)
<i>lf.n.</i> , labro-frontal nerve	I, cell group I
<i>m.b.</i> , mushroom body	II, cell group II
<i>md.l.</i> , mandibular lobe	III, cell group III
<i>md.n.</i> , mandibular nerve	IV, cell group IV
<i>m.f.</i> , middle fiber mass	
<i>oc.</i> , ocellus	
<i>oe.</i> , oesophagus	<i>Fiber tracts</i>
<i>o.b.</i> , optic body	<i>b</i> , tract b
<i>o.l.</i> , optic lobe	<i>c</i> , tract c
<i>o.n.</i> , ocellar nerve	<i>cx</i> , tract ex
<i>o.o.l.</i> , } outer ocellar lobes	<i>d</i> , tract d
<i>o.oc.l.</i> , }	<i>e</i> , tract e
<i>o.f.</i> , outer fiber mass	<i>f</i> , tract f
	<i>fl</i> , tract fl
	<i>l</i> , tract l
	<i>m</i> , tract m
	<i>n</i> , tract n
	<i>p</i> , tract p
	<i>r</i> , tract r

PLATE 1

EXPLANATION OF FIGURES

Figures 1 to 9 are drawn from whole mounts of the heads. The nerve cell layer is stippled, the fibrous core blank. Oc. 2, obj. AA, Zeiss, table-level, reduced one-half.

- 1 The brain of the queen of *Camponotus pennsylvanicus*.
- 2 The head and brain of the worker of *C. pennsylvanicus*.
- 3 The head and brain of the male of *C. pennsylvanicus*.
- 4 The head and brain of the queen of *Formica schaufussi*.
- 5 The head and brain of the worker of *F. schaufussi*.
- 6 The head and brain of the male of *F. schaufussi*.
- 7 The head and brain of the queen of *Lasius niger*.
- 8 The head and brain of the worker of *L. niger*.
- 9 The head and brain of the male of *L. niger*.

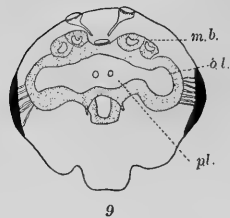
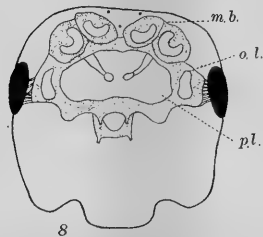
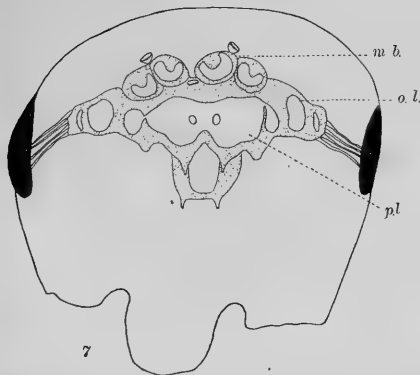
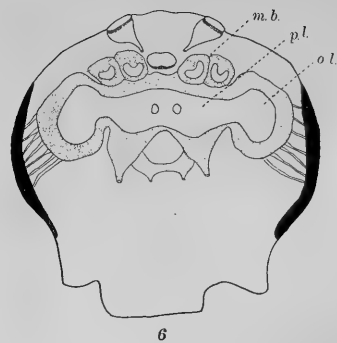
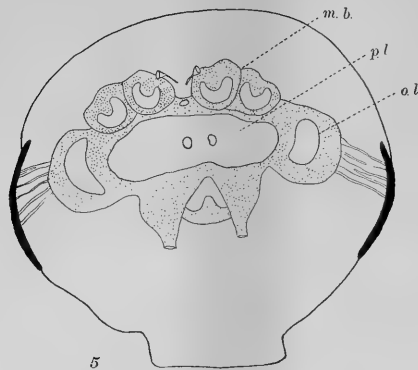
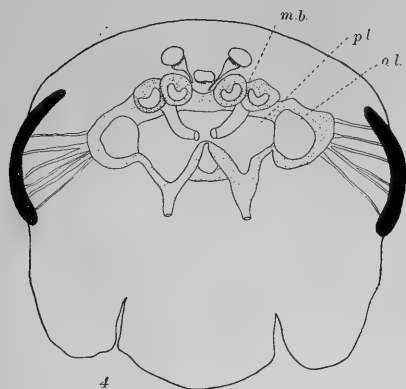
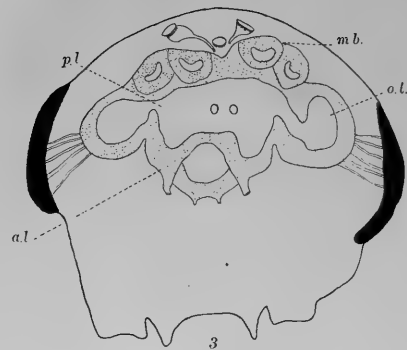
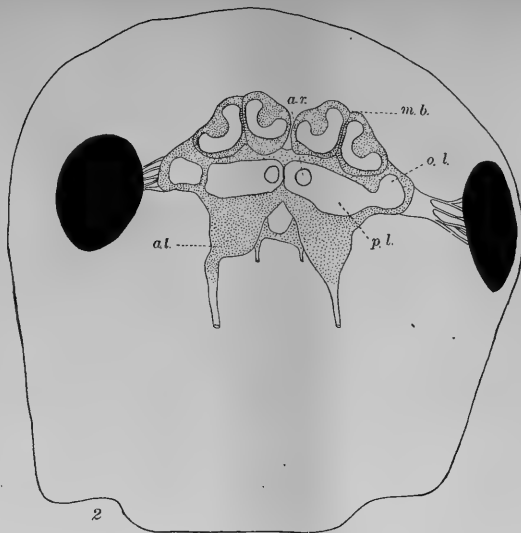
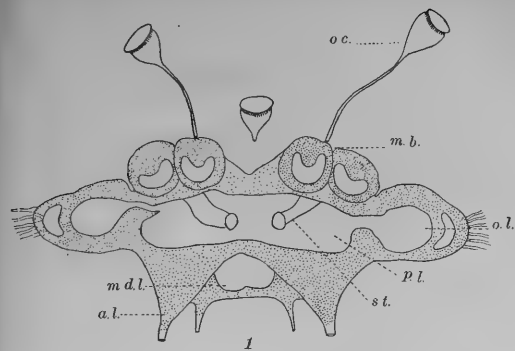


PLATE 2

EXPLANATION OF FIGURES

Figures 10 to 16 are taken from a series of frontal sections of the head of the queen of *Lasius niger*, beginning at the anterior end. The fibrous core is mottled, the nerve cell layer is blank. Oc. 4, AA, Zeiss, stage level.

10 Section through the anterior part of the protocerebral lobes, *p.l.*, and the antennary lobes, *a.l.*, anterior to the mushroom bodies.

11 Section through the anterior part of the mushroom bodies, and showing the origin of the labro-frontal nerve, *l.fn.*, from the antennary lobe.

12 Section through the stalks, *st.*, of the mushroom bodies, and the anterior dorsal protocerebral commissure, *a.cm.*

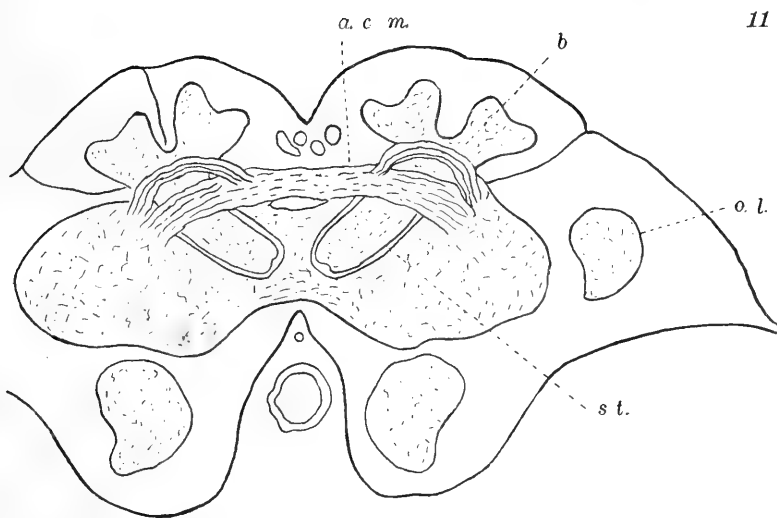
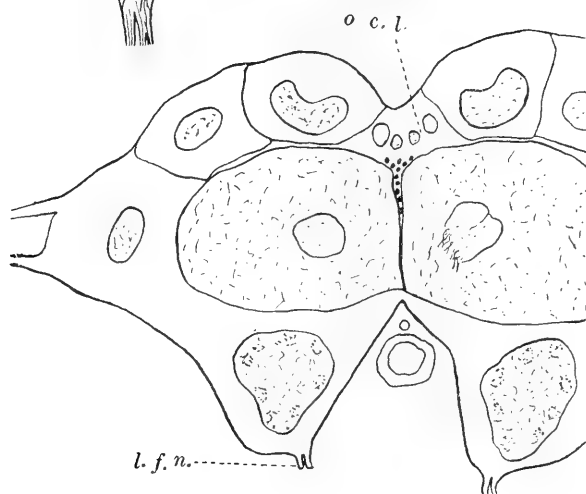
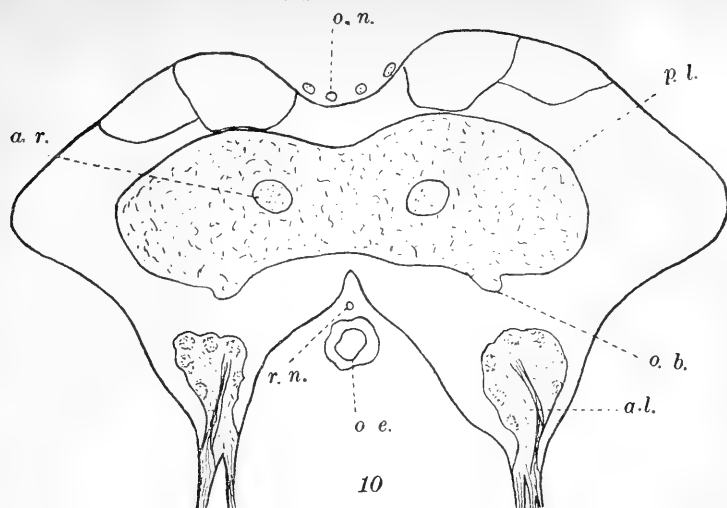


PLATE 3

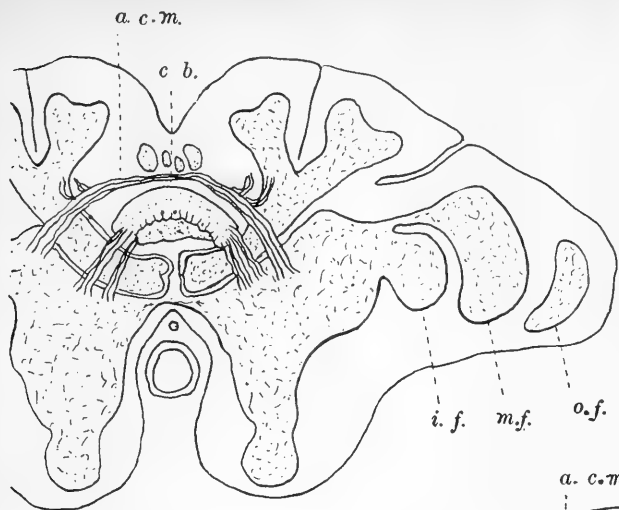
EXPLANATION OF FIGURES

13 Section showing the central body, *cb.*, the mushroom bodies, the optic lobes, and the anterior dorsal commissure, *a.cm.*

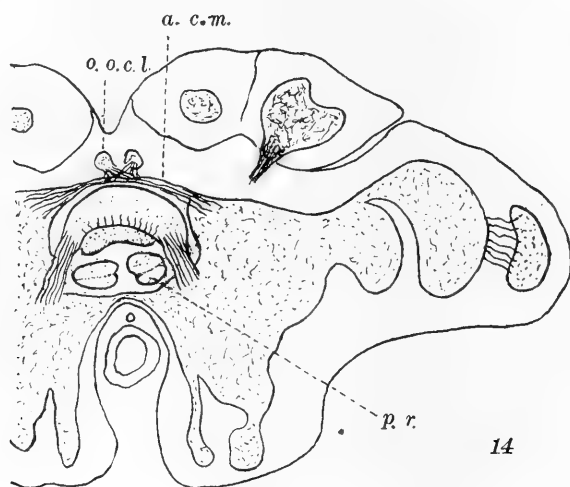
14 Section showing the distal ends of the mushroom body stalks beneath the central body, and the division of these stalks into two parts. The ventral parts, *p.r.*, represent the posterior roots of the mushroom bodies. Fibers are seen passing from the outer ocellar lobes, *o.oc.l.*, into the anterior dorsal commissure, *a.cm.*

15 Section through the posterior end of the protocerebral lobes, where these are united with the subesophageal ganglion, showing the posterior roots of the mushroom bodies, *p.r.*, and the posterior dorsal protocerebral commissure, *p.cm.*

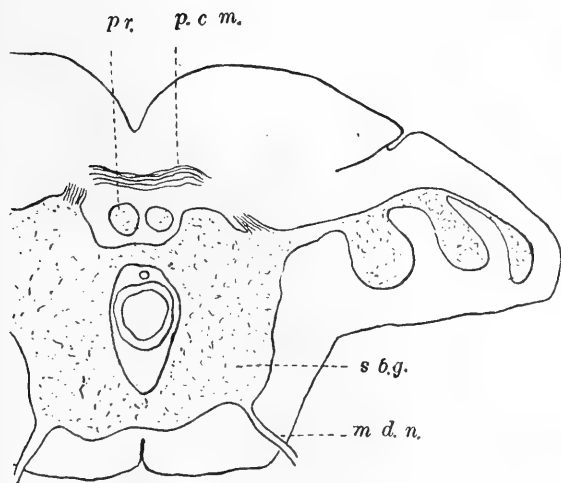
16 Section showing the subesophageal ganglion, the labral nerve, and the trito-cerebral lobes and nerves, *tr.l.*, *tr.n.*



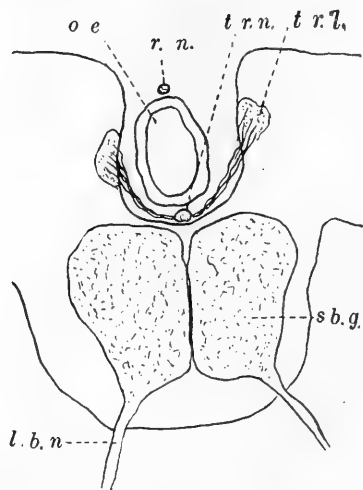
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PLATE 4

EXPLANATION OF FIGURES

Figures 17 to 20 are consecutive sections from a frontal series of the head of the queen of *Lasius niger*; figure 21 is two sections behind figure 20. Oc. 4, obj. AA, Zeiss.

17 Section showing the fibrous core of the protocerebral lobes, the anterior dorsal commissure, *a.cm.*, the outer ocellar lobes, *o.oc.l.*, whose fibers pass into the commissure, and the distal ends of the stalks of the mushroom bodies, *st.*, partly divided into two bundles.

18 Section showing the complete division of the stalks into two bundles, two dorsal bundles, *d.b.*, fibers from which are entering the central body, and two ventral bundles, the posterior roots of the mushroom bodies, *v.b. (p.r.)*. The anterior commissure is no longer present.

19 Section showing the entrance of fibers from the dorsal bundles into the central body, *c.b.*, which is disappearing.

20 Section showing the central part of the posterior commissure, *p.cm.*, and the posterior roots, *p.r.*, which are about to enter the protocerebral fibrous core.

21 Section showing the lateral ends of the posterior commissure, *p.cm.*, and the posterior roots, *p.r.*, now within the fibrous core.

22 *Camponotus* male. Section showing the posterior commissure, *p.cm.*, the posterior roots, *p.r.*, within the protocerebral fibrous core, and the ocellar nerve fibers passing down from the four ocellar lobes. Oc. 4, obj. AA, Zeiss.

23 *Formica*, queen. Section showing the entrance of fibers from the outer ocellar lobes, *o.oc.l.*, down through the posterior commissure, *p.cm.* Oc. 4, Obj. AA, Zeiss.

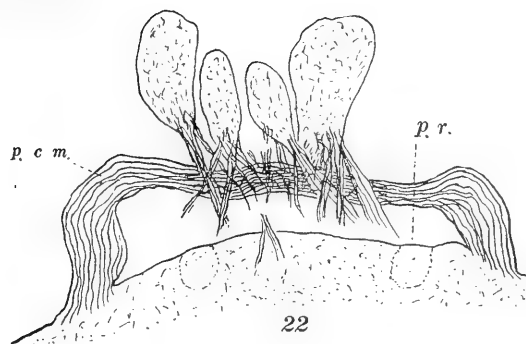
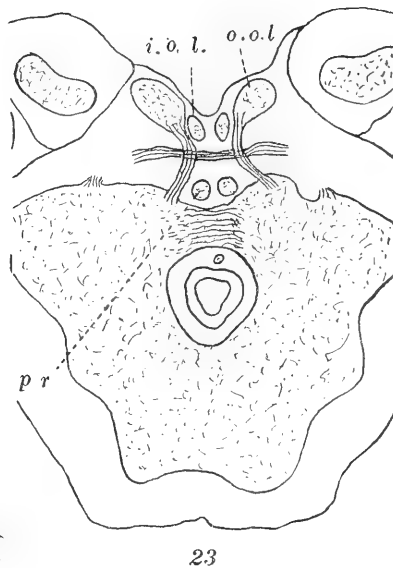
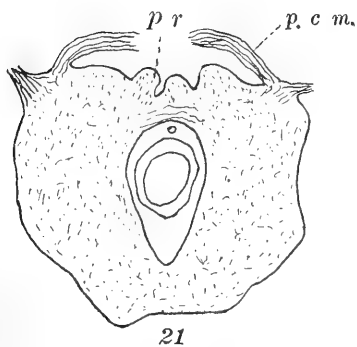
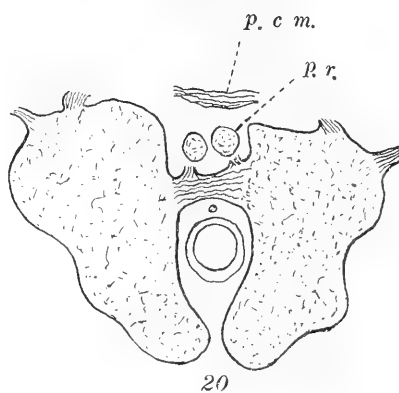
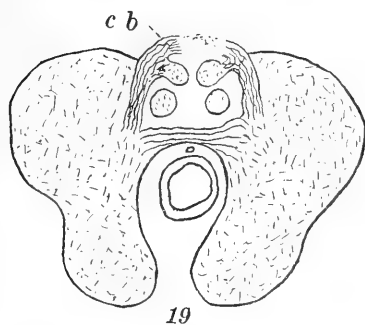
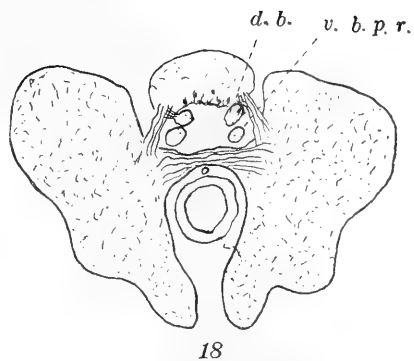
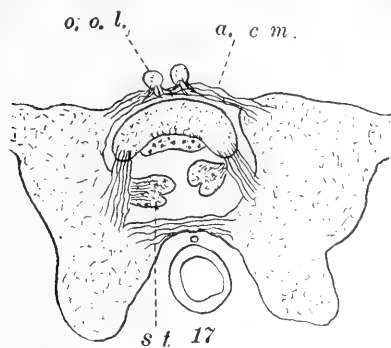


PLATE 5

EXPLANATION OF FIGURES

24 Camponotus queen. Anterior root of the mushroom body, showing the division into four bundles of transversely cut fibers. Oc. 2, obj. D, Zeiss.

25 Camponotus queen. Anterior root of the mushroom body divided into two bundles, and showing fibers passing to and from the protocerebral core. Oc. 2, obj. D, Zeiss.

26 Camponotus queen. Section of the stalk of a mushroom body showing the 'decussation,' or origin of the anterior root fibers. Oc. 2, obj. D, Zeiss.

27 Camponotus worker. Cells from Groups I, II, III, IV of the mushroom bodies. Homog. immers. 1/12, oc. 2, Zeiss.

28 Camponotus queen. Part of the frontal ganglion. Homog. immers. 1/12, oc. 2, Zeiss.

29 Lasius worker. The nuclei of the cells of one lobe of a mushroom body, showing the Groups I to IV. Oc. 2, immers. 1/12, B. and L.

30 Camponotus queen. Cells composing the brain sheath. Homog. immers. 1/12, oc. 2, Zeiss.

31 Camponotus worker. A nerve cell from the intercerebral region. Homog. immers. 1/12, oc. 2, Zeiss.

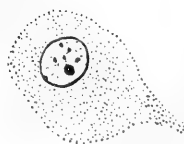
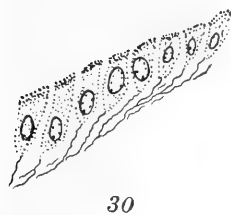
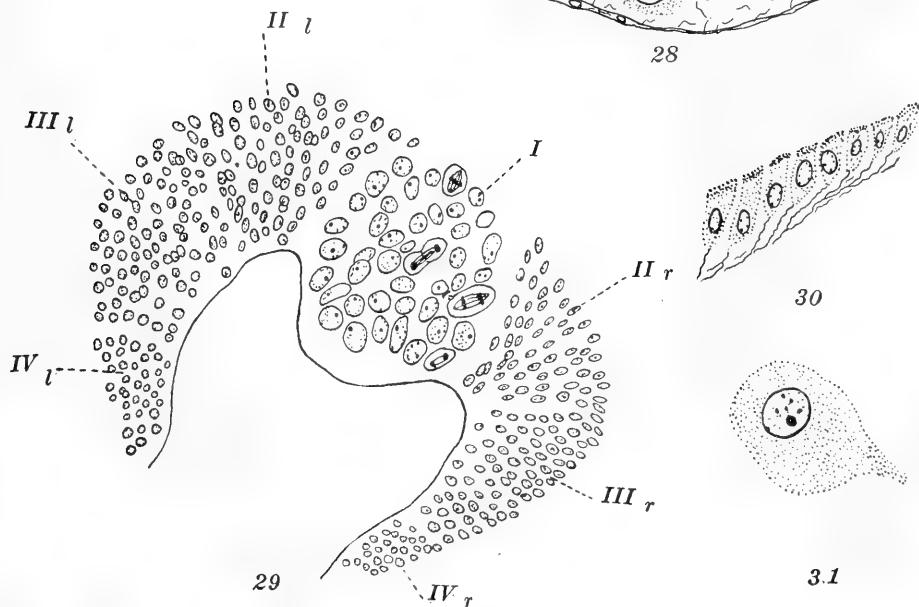
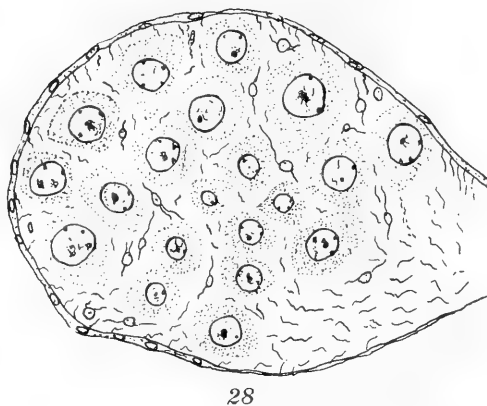
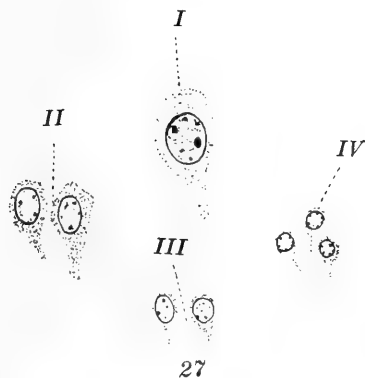
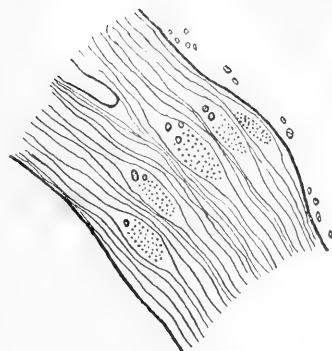
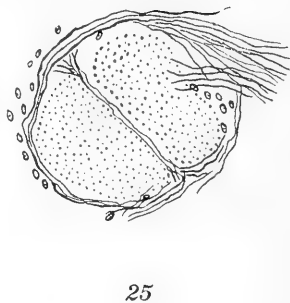


PLATE 6

EXPLANATION OF FIGURES

The outlines of figures 32 to 40 were drawn with the camera, but the fiber tracts are reconstructed from drawings of other sections.

These figures, therefore, represent diagrams of the mushroom body fiber tracts as if seen in optical section.

32 The fiber tracts of the mushroom bodies of the queen of *Camponotus pennsylvanicus*.

33 The fiber tracts of the mushroom bodies of the worker of *C. pennsylvanicus*.

34 The fiber tracts of the mushroom bodies of the male of *C. pennsylvanicus*.

35 The fiber tracts of the mushroom bodies of the queen of *Formica schaufussi*.

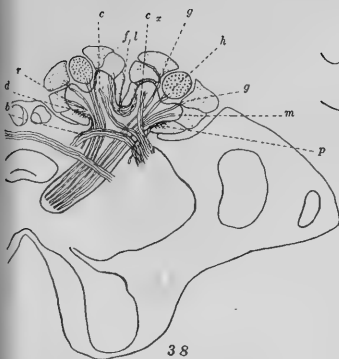
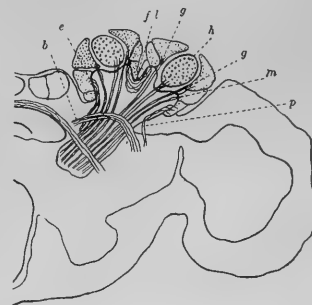
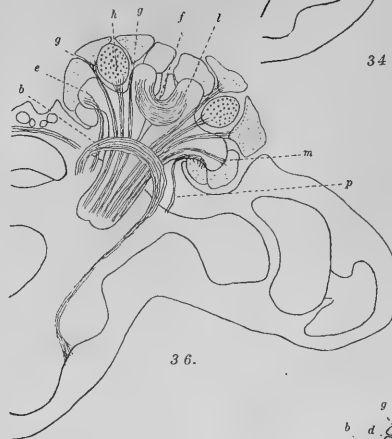
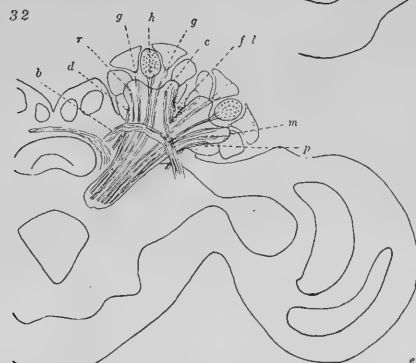
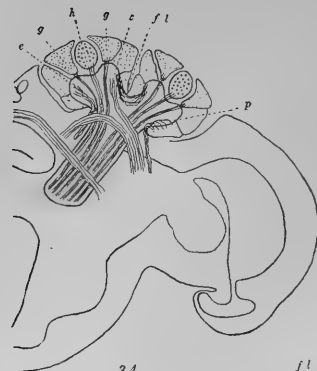
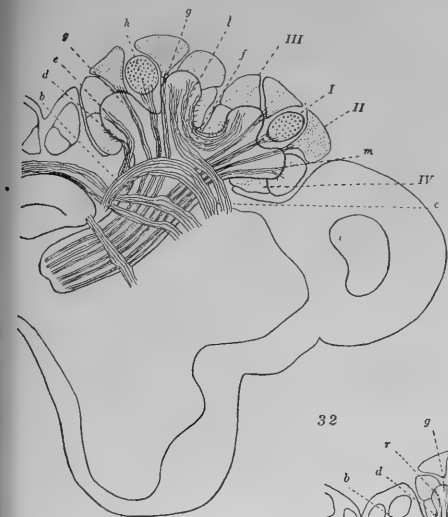
36 The fiber tracts of the mushroom bodies of the worker of *F. schaufussi*.

37 The fiber tracts of the mushroom bodies of the male of *F. schaufussi*.

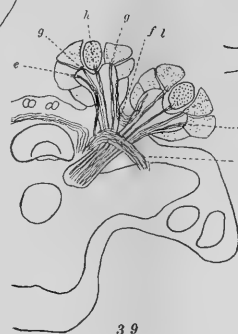
38 The fiber tracts of the mushroom bodies of the queen of *Lasius niger*.

39 The fiber tracts of the mushroom bodies of the worker of *Lasius niger*.

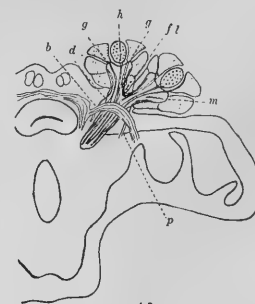
40 The fiber tracts of the mushroom bodies of the male of *Lasius niger*.



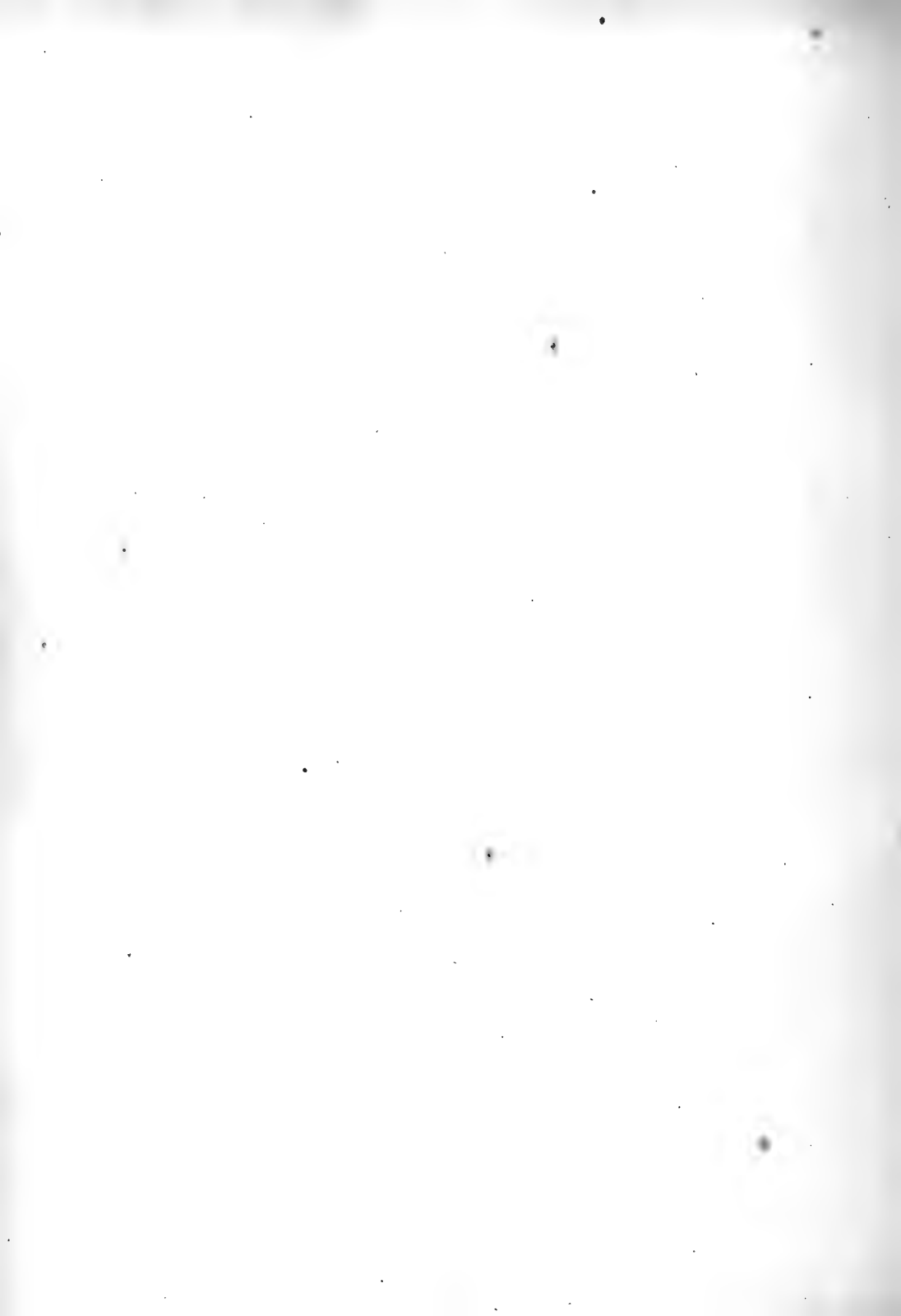
35



39



40



THE ORIGIN OF THE LATERAL LINE PRIMORDIA IN LEPIDOSTEUS OSSEUS¹

F. L. LANDACRE AND A. C. CONGER

THIRTY-FOUR FIGURES

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¹Contributions from The Department of Zoology of the Ohio State University, No. 37.

INTRODUCTION

The lack of agreement among workers as to the relation of the lateral line primordia—that is, the lateral sensory ridges preceding the definite lateral line organs—to the auditory vesicle, is sufficiently marked to require a re-examination of the whole question. Part of this lack of agreement may be due to differences in the types studied, but as a whole it can hardly be explained in this way.

H. V. Wilson ('91) and a number of other workers trace the primordia of the lateral line organs directly to the auditory vesicle and describe the earliest stages of these primordia as anterior and posterior extensions of the same epidermal thickening that gives rise to the auditory vesicle. On the other hand, Platt ('95-'96), Clapp ('99), Beckwith ('07) and Landacre ('10) find no such connection between the primordia of the sensory lines and the vesicle, but find the primordia arising close to the vesicle but independent of its anterior and posterior extensions.

Landacre ('10) found in *Ameiurus* no extended sensory ridges preceding the appearance of definitive lateral line organs, although several organs may arise sometimes close together from a short epidermal thickening. He also traced the degeneration of the anterior and posterior extensions of the auditory vesicle before the lateral line organs appeared. *Ameiurus* thus furnishes striking evidence for the independent origin of the lateral line organs and auditory vesicle.

Since, however, in *Ameiurus* the lateral line organs arise independently and are not preceded by lateral sensory ridges, it seems desirable to go carefully over the origin of these structures in some type which has well defined sensory lines preceding the appearance of definitive lateral line organs to determine, if possible, the exact relation of the lateral line primordia to the auditory vesicle; and to determine further, if these primordia arise independently, what embryological structures are so situated as to lead workers to infer a relationship between them that may not exist.

Lepidosteus osseus was chosen for this study on account of its systematic position, and particularly on account of the fact that it has well defined sensory lines preceding the appearance of lateral line organs.

HISTORICAL REVIEW

The papers of Froriep ('85) and of Beard ('85 '88) while they do not bear directly on the relation of the sensory lines to the auditory vesicle, are still of interest in view of the variety of structures lying in the preauditory region that might be mistaken for primordia of sensory lines.

Froriep ('85) described in mammals what he called "Anlagen of sense organs" on the facialis, glossopharyngeus and vagus and thought that their relationship placed them with the lateral line system. When we recall the fact that lateral lines are not present in mammals and find that the structures he figures and describes as "sense-organ Anlagen" lie just above the gill clefts, we assume that they were in reality the epibranchial placodes.

Beard ('85 '88) confirmed the observations of Froriep and described similar epidermal thickenings in reptiles, birds, teleosts and elasmobranchs, and like Froriep thought they were related to the lateral nerves. He introduced the term "branchial sense organs" for these primordia which may have been primordia of the lateral line but seem, in most if not all cases, to have been epibranchial placodes, on account of the relation to the gill slits which he attributes to them. He failed, however, to make a clear distinction between dorso-lateral and ventro-lateral placodes, as von Kupffer ('91) points out, nor can we find in Beard's writings a consistent distinction between the dorso-lateral placodes and the early stages of the lateral line organs. The distinction between dorso-lateral placodes and ventro-lateral or epibranchial placodes is clearly stated by von Kupffer ('91) and he seems clear as to the relation of the epibranchial placodes to the visceral ganglia, rather than to the so-called lateral nerves.

The work of Froriep and Beard throws little light on the problem of this paper further than to make it clear that there are

other epidermal thickenings that might be confused with those concerned in the formation of the lateral line organs. It is quite evident that no definite conclusion can be reached until we know exactly what is to be included, or rather what is to be excluded, in the genesis of this system. This means that we must carefully exclude those placodes concerned in the formation of visceral ganglia, the epibranchial placodes, and probably those concerned in the formation of lateralis ganglia as distinct from lateral line organs, one or both of which these authors included in the formation of the lateral line primordia. As the text of this paper will show, there are still other epidermal thickenings which may be easily taken for lateral line primordia but which do not belong with this system at all.

Ayers ('92) in commenting on the conclusions of Beard mentioned above, makes the following statement: "As Froriep has shown the ectodermal thickenings which Beard described as having given rise to the lateral line organs have in fact another fate. The genuine lateral line organs escaped Beard's attention." Locy ('95) states that the branchial sense organs of Beard and Froriep are not lateral line organs. Cole ('98, p. 151), in his work on *Gadus*, follows Ayers' criticism of the conclusions of Beard (as cited above) with the following remark: "It is possible that his (Ayers) latter statement is to some extent sufficiently near the truth to require a re-opening of the whole question of the 'branchial' or 'epibranchial' sense organs."

Among the papers taking up specifically the relations of the lateral line primordia to the ear must be mentioned those of Mitrophanow and Wilson. Mitrophanow ('91) described a common primordium for the lateral line system and the auditory vesicle. In his observations on the lateral line system of elasmobranchs ('93), he repeats his first statement, and says that the auditory pits and the sense organs of the lateral line system have a common origin in a thickened band of epithelium. The auditory vesicle separates from the sensory band, which is thus cut into two portions; the anterior portion, lying in the region of the fifth nerve and corresponding to Beard's "branchial sense-organ," gives rise to the supra- and infra-orbital lines, while the posterior

portion, found in the region of the ninth nerve, later forms the lateral line posterior to the auditory vesicle.

Wilson ('91) in his paper on *Serranus*, traces the lateral lines and the auditory organ back to a common sensory furrow, which (p. 244) gives rise not only to the ear, but to a "functional branchial sense organ," and to the organs of the lateral line as well. This furrow begins to deepen at two points, where the auditory organ and the branchial sense organ will be formed. The process of invagination continues until there are two well-marked sacs, the furrow persisting between them and continuing for some distance behind the auditory sac. At a still later stage, the three derivatives (branchial sense organ, auditory sac, and lateral line primordium) have moved away from each other. The (first) gill-slit breaks through and just in front of it is the branchial sense organ, the auditory sac is overgrown by the medulla, and the primordium of the lateral line moves back some distance from its original position in front of the somites. Later, "the lateral line anlage has grown still farther back, and is incompletely divided into three sense organs of the lateral line."

Concerning the question at issue, that is, the relation of the lateral line primordia to the auditory vesicle, Wilson is very definite. It is unfortunate, however, that he perpetuated Beard's term "branchial sense organ." Beard thought it was a part of the lateral line system but concerned in some way with the function of the gills. Since Wilson goes no further than to characterize it as a 'functional' branchial sense organ, we are left to choose between lateral line primordia, epibranchial placodes, thickenings at the point of contact of endoderm of gill with ectoderm, and preauditory placode (Landacre '10). The last seems the more probable interpretation and, if correct, the term branchial sense organ should be dropped.

Wilson and Mattocks ('97) confirm in the salmon the previous work of Wilson in the sea bass, as to the common primordium of the lateral sense organs and the auditory organ, and further state that the portion in front of the auditory vesicle (branchial sense organ of Beard) gives rise by bifurcation to the supra-orbital and infra-orbital lines, while the portion posterior to the

auditory vesicle grows backward and forms the lateral line. Loey ('95) confirms in the type *Squalus* the observations of Wilson in *Serranus* and *Mitrophanow* in *Acanthias* as to the common primordium of the lateral sense organs and the auditory organ.

On the other hand, a number of authors after making a careful study of the relation of the lateral line system to the ear have been unable even with the preceding results in mind to find any genetic relation between the primordia of the lateral line organs or the organs themselves and the ear. These results are especially significant in the light of the conclusions found in *Lepidosteus*, where the authors feel that they have not only demonstrated that there is no genetic connection between the two but have found ample grounds for explaining the contradictory results obtained in other types provided they resemble *Lepidosteus* at all closely.

Miss Platt ('95) cleared up much of confusion arising from the investigations of former workers by making a sharp distinction between the two series of placodes, dorsolateral and epibranchial, on the one hand, and the early stages of the sensory lines on the other hand. She points out the fact that sensory lines may appear later in the exact place occupied by a placode or ectodermal thickening, and further states that there is a resemblance between the growing point of a placode and a lateral line organ.

Miss Clapp ('99) does not trace the sensory lines in *Batrachus* to the auditory vesicle or its derivatives, although she does state that they originate in the region of the auditory vesicle. Dr. Clapp's conservative statement is significant in view of the amount of time she spent in an attempt to trace the lateral sensory line and the auditory vesicle to the same primordium in *Batrachus*.

Miss Beckwith ('07) investigated the development of the lateral line system in *Amia* from its first appearance up to the first stage described by Allis ('89). She states that from her observations

It is evident that the lateral line anlagen do not arise in connection with the auditory organ as described in teleosts (Wilson '91). The auditory organ arises before the lateral line and independently of it, as an in-

vagination of the nervous layer of the ectoderm, and forms later a closed vesicle. The lateral line appears first in an embryo in which the auditory organ is a closed vesicle. The four primary lines (supra-orbital, infra-orbital, hyo-mandibular and body lines) arise independently of one another and of the auditory organ as thickenings of the nervous layer of the ectoderm.

Landacre ('10) was the first to apply the term "preauditory placode" to the anterior extension of the auditory vesicle from the point where the vesicle narrows down at its future anterior boundary to the extreme anterior limit of the extension. He states (p. 339) that in *Ameiurus* for a time the preauditory placode ('branchial sense organ' of Wilson and Beard?) simulates in appearance a lateral line organ, and then later disappears, without giving rise to lateral line organs, there being a period of more than twenty-six hours between the disappearance of the preauditory placode and the appearance of the lateral line organs. In the region posterior to the auditory vesicle he finds that the postauditory placode loses the characteristic cell arrangement, and although the lateral line organs appear along the route traversed by the placode as it moves back, they are not derived from the postauditory placode; further the lateral line organs in *Ameiurus* appear as individual differentiations of the ectoderm and are not preceded by a continuous line in *Ameiurus* as in other types described. In a paper two years later ('12) he describes the preauditory placode as disappearing and fails to find any genetic relation between the preauditory placode and the sensory lines.

From the preceding brief review of the literature it is evident that there are two quite divergent points of view. The first holds that the lateral line primordia, that is, the sensory lines found in approximately the positions in which the supra-orbital, infra-orbital, mandibular and body lines are later situated, are derived from and continuous at first with the epidermal thickening out of which the auditory vesicle forms.

The second explanation starts with the same primordia, the auditory vesicle and its anterior and posterior prolongations (pre- and postauditory placodes) on the one hand, and on the other the primordia of the lateral lines, namely, the sensory lines; but

does not trace the latter to the auditory vesicle or its anterior or posterior extensions but finds them originating separately and distinct from those structures. The auditory vesicle and its anterior and posterior extensions appear first and are followed by the sensory lines along the course of which the lateral line organs differentiate, or as in the case of *Ameiurus*, even by the appearance of individual lateral line organs which are not preceded by sensory lines.

MATERIAL AND METHOD

The material used in this investigation consists of series of *Lepidosteus osseus*, taken from one lot of eggs at six-hour stages, and fixed in Zenker's fluid. Although the interval is long, an examination of a number of individuals shows sufficient variation in the degree of development to make the series practically continuous. The sections were cut $6\ \mu$ thick and stained in bulk for twenty-four hours in Delafield's hematoxylin, one-sixth the strength of stock solution.

Table 1 shows the age, increment in hours, the length and increment in length wherever possible. Stages X to XVII are referred to in the discussion by age only, since in these stages the embryos were not removed from the membranes before fixation and consequently accurate measurements cannot be given.

The method employed has been to locate the sensory lines in an advanced stage (stage XXVI) and then trace these structures back through development to their earliest recognizable stages. Since it has been the purpose to find, if possible, the relation of the preauditory placode (Landacre '10, 'branchial sense organ' of Wilson and Beard) to the sensory lines, we have traced the development of the preauditory placode from the point where it is continuous with the auditory vesicle up to the last stage where it can be positively identified. The history of the ectodermal thickenings in the region where the endoderm of the hyoid gill pocket approaches the epidermis have also been carefully studied, since their size in this type renders them important factors in this investigation.

THE 10-MM. EMBRYO

Before taking up the problem of the origin of the lateral lines it seems advisable to give a brief description of some advanced stage of the lateral lines. For this we have chosen the 10-mm. embryo which is the same as that plotted by Landacre ('12). Plate 1 of his paper should be consulted for a general description of the nerves and ganglia of a 10-mm. embryo. The three sensory lines of the head are present at this stage (fig. 6 of the present paper). The supra-orbital line extends from a point about midway between the optic vesicle and the auditory vesicle cephalad and dorsad in a gentle curve until it ends in the region immediately dorsal to the anterior end of the optic vesicle. The cells

TABLE 1

*Showing age, length, increment in age and length of embryos of *Lepidosteus**

STAGES	AGE FROM FERTILIZATION, IN HOURS	INCREMENT, IN HOURS	LENGTH, IN MM.	INCREMENT IN LENGTH, IN MM.
X.....	54	6		
XI.....	60	6		
XII.....	66	6		
XIII.....	72	6		
XIV.....	76	4		
XV.....	82	6		
XVI.....	88	6		
XVII.....	94	6		
XVIII.....	100	6	7.0	
XIX.....	106	6	7.3	0.3
XX.....	112	6	8.0	0.7
XXI.....	120½	8½	8.3	0.3
XXII.....	130	9½	8.8	0.5
XXIII.....	137	7	9.5	0.7
XXIV.....	144	7	9.7	0.2
XXV.....	148	4	9.9	0.2
XXVI.....	154	6	10.0	0.1
XXVII.....	160	6	10.9	0.9
XXVIII.....	166	6	11.3	0.4
XXIX.....	172	6	11.5	0.2
XXX.....	184	12	12.4	0.9
XXXI.....	191	7	12.9	0.5
XXXII.....	196	5	13	0.1

of the line show the characteristic radial arrangement of the sensory lines much more plainly at some points than at others. These points mark the positions of future lateral line organs. The infra-orbital line begins behind and ventral to the posterior end of the supra-orbital line, and runs cephalad and ventrad in a curve which follows, in general, the outline of the optic vesicle. The infra-orbital line ends anteriorly at a point ventral to the anterior end of the optic vesicle and at the level of the ventral border of the olfactory capsule.

Owing to the angle of sectioning, the cells of the mandibular line do not exhibit the characteristic radial arrangement in so marked a degree as the cells of the supra-orbital and infra-orbital lines. The mandibular line begins in the region of the hyoid gill and may be traced cephalad and sharply ventrad until it is lost in the thickened epithelium where the embryo joins the yolk sac.

No trace of an anastomosis could be noted at this stage, either between the supra-orbital or sub-orbital lines, or between these and the mandibular line.

In the postauditory region of the 8.5 mm. embryo there are three lateral line primordia. The first and most anterior is not strictly postauditory in position but lies in the region of the posterior third of the auditory vesicle and its organs are innervated by the ramus supratemporalis X. The second extends forward from the anterior end of the lateralis X ganglion and in a vertical axis lies over the second gill and its organs are innervated by the ramus supratemporalis X. The third extends from the posterior end of the lateralis X ganglion toward the posterior end of the body. Its organs are innervated by the main lateral line ramus of the X ganglion.

EARLY STAGES

No attempt has been made to trace carefully the very early stages in the development preceding the appearance of the primordium of the auditory vesicle and the preauditory placode, since that problem is not within the scope of this paper as it involves the origin of cranial ganglia not related to the sensory

lines. Therefore, only a cursory examination was made up to the point where well developed primordia of the auditory vesicle and preauditory placode can be recognized.

The description of the relation of the auditory vesicles to the lateral line primordia begins naturally with the earliest trace of these structures that can be definitely identified. This is, of course, the primordium of the auditory vesicle, since this appears some hours before any trace of the lateral line primordia can be found. The earliest stages are not figured, since these contain nothing that bears directly on the relation of the two structures in question.

In a 54-hour stage, a lateral thickening of the ectoderm shows the first evidence of the formation of the auditory vesicle and the preauditory placode. This thickening may be traced for a considerable distance parallel to the infolded neural tube. The 60-hour stage and the 66-hour stage show only an increase in development, in that the cells in the lateral thickening exhibit a more marked radial arrangement, more of the cell nuclei come to occupy positions in the distal ends of the cells, and the whole cord, which is the primordium of the auditory vesicle and the preauditory placode, becomes thicker and more prominent.

A 72-hour stage (figs. 23 to 26) shows, for the first time, the auditory vesicle becoming detached from the ectoderm. The process of detachment begins at the posterior limit of the vesicle and moves cephalad, the vesicle coming to occupy a position at some distance from the epidermis, but connected with it by strands of drawn-out cells and cytoplasm. It should be noted here that the posterior end of the auditory vesicle is not extended backward into a postauditory placode and that, as stated, when it becomes free from the ectoderm it ends in a rounded knot caudad. This condition will be described in detail in the second part of the paper on the postauditory region. Its bearing on the theory of the origin of the postauditory or body lateral line is of the greatest importance, since the absence of a posterior extension of the auditory vesicle removes from the field of controversy the question as to the origin of the primordium of the postauditory or body line from the auditory vesicle in *Lepidosteus*.

The ectodermal thickening of the first true gill is present in this stage. It lies ventro-laterally from the auditory vesicle (figs. 24, 25, *Ec.Th.1.*). Its total length is $150\ \mu$ and it is coextensive with the attachment of the auditory vesicle to the ectoderm. The anterior third of the thickening lies in the area of contact of the pharyngeal pocket of the first true gill with the ectoderm and two thirds of the thickening extends posterior to the gill contact as a thickened cord in the ectoderm. It was mistaken at first for a postauditory placode, but its later history shows it to be a true gill thickening comparable with the similar thickenings on the VII and the other four true gills.

In this stage the radially arranged cells of the preauditory placode (anterior extension of the auditory vesicle) can be recognized extending forward to within three sections of the posterior limit of the actual contact of ectoderm in the region of the hyoid gill pocket. In the description of later stages we shall use as a land-mark the last section which shows a connection between the ectoderm and endoderm in the region of this gill-pocket, and structures will be located by the number of sections posterior to the point of 'hyoid contact.'

In the 72-hour stage at a point seven sections ($70\ \mu$) posterior to the hyoid contact the preauditory placode resembles the auditory vesicle except in size. Cephalad of this point it does not resemble the vesicle closely and becomes less prominent towards its anterior end. The radial arrangement of the cells is very pronounced and the placode has become so thick that it projects well beyond the inner surface of the ectoderm. There is no sharp differentiation between the preauditory placode and the auditory vesicle, and one reads back without break in continuity of structure directly into the true vesicle. Twenty-five sections posterior to the hyoid contact, the cavity of the vesicle may be recognized, and eight sections beyond this, or $330\ \mu$ posterior to the hyoid contact, the vesicle is detached from the ectoderm.

In a 76-hour stage, the anterior end of the preauditory placode has moved back one section from its position in the preceding stage, or four sections posterior to the hyoid contact. There is still histologically no sharp differentiation between the preaudi-

tory placode and the auditory vesicle (fig. 17). The cavity of the vesicle is $270\ \mu$ posterior to the hyoid contact and the anterior point of detachment is $100\ \mu$ beyond the cavity, or $370\ \mu$ posterior to the hyoid contact.

In the postauditory region the thickening of the epidermis mentioned in the description of the 72-hour stage (figs. 24, 25, *Ec. Th.1.*) has changed slightly. It extends somewhat posterior to the point of contact of the endoderm of the gill-pocket with the ectoderm and toward its posterior end is somewhat broader in its dorso-ventral axis (fig. 27), a condition which will be shown later to be associated with the appearance of a second thickening of the epidermis associated with the second true gill and the first epibranchial placode of this gill.

THE PREAUDITORY REGION

The presence of five closely associated structures in the region between the auditory vesicle and the optic vesicle renders difficult the determination of the mode of origin of the sensory lines anterior to the auditory vesicle. These five structures are the preauditory placode (Landacre '10; 'branchial sense organ' of Wilson and Beard) the ectodermal thickening at the point where the hyoid gill pocket touches the epidermis, the epibranchial placode, the posterior extension of the epibranchial placode and the primordia of the lateral lines.

The structures named, with the exception of the posterior extension of the epibranchial placode, are shown in figure 2, which represents the 94-hour stage. In this stage the preauditory placode is $70\ \mu$ in length and extends from a point $30\ \mu$ in front of the anterior limit of the auditory vesicle to a point $40\ \mu$ behind the anterior limit of the vesicle.

The hyoid ectodermal thickening extends from a point $130\ \mu$ in front of the anterior boundary of the auditory vesicle and $30\ \mu$ behind the posterior limit of the actual contact of the ectoderm and endoderm where the hyoid gill pocket touches the epidermis, to a point $20\ \mu$ in front of the anterior limit of the actual contact. The total length of the ectodermal thickening in this stage is

190 μ , while the actual contact is 140 μ in length (the contact is shown in figure 2 by an unshaded line).

The epibranchial placode, which is first recognizable in this stage, occupies the region immediately behind the posterior limit of the thickening of the ectoderm in the region where the hyoid gill pocket approaches the epidermis. The epibranchial placode is 30 μ in length in this stage and its long axis lies in a dorso-ventral plane.

The posterior extension of the epibranchial placode, which does not appear in the 94-hour stage, will occupy the region just posterior to the epibranchial placode (fig. 3), and will lie at a lower level than that occupied by the preauditory placode. During the maximum development of the posterior extension it may be recognized for a distance of four or five sections behind the epibranchial placode.

Since these four structures play so important a part in the study of the pre-auditory region, we shall take them up in order, and trace the history of each up to a stage when well-developed sensory lines can be recognized.

a. Preauditory placode

Of the structures named above, the first two, the preauditory placode and the ectodermal thickening, appear at very early stages and it would be difficult to state definitely whether one precedes the other or not. The mode of development of the early stages of the auditory vesicle and the preauditory placode, as observed by the writers, agrees in general with the account by Wilson ('91) in Serranus. In very early stages a thickened cord of cells may be found lying along the neural cord and especially pronounced in the region marked later by the appearance of the auditory vesicle. The central portion invaginates, becomes detached, and forms the auditory vesicle. The anterior portion, which is the primodium of the preauditory placode, persists after the auditory vesicle has moved in from the epidermis and assumed its rounded form. The cells of the placode show its relationship to the auditory vesicle by exhibiting the same radial arrangement, which is so marked a character of the auditory vesicle.

In a 76-hour stage (fig. 17) the preauditory placode appears as a cord of cells which may be traced cephalad from the anterior end of the auditory vesicle to a point $40\ \mu$ posterior to the contact of the ectoderm and endoderm at the hyoid gill pocket.

Six hours later, the 82-hour stage, shows a slight modification of the anterior end of the preauditory placode in the partial loss of the radial arrangement of the cells. The placode (fig. 19) is in contact with a posterior extension of the ectodermal thickening extending caudad from the point where the hyoid gill pocket comes into contact with the epidermis, but in this stage $50\ \mu$ intervene between the anterior end of the preauditory placode and the extreme posterior limit of the actual contact of ectoderm and endoderm. In addition there is the histological difference between the preauditory placode which retains some indication of the radial arrangement of its cells and the hyoid ectodermal thickening which does not show any indication of such arrangement. In the 88-hour stage (figs. 1 and 22) the preauditory placode is further shortened, the cells at the anterior end losing their characteristic radial arrangement so that it can be traced forward now only to a point $70\ \mu$ posterior to the actual contact. It should be noted, however, that there is a thickening of the epidermis extending from the posterior limit of the hyoid contact back to the anterior end of the preauditory placode, but it does not show a radial arrangement of cells, as does the preauditory placode.

The last trace of the preauditory placode is found in the 94-hour stage (fig. 2). In this stage the placode has lost its connection with the auditory vesicle, but overlaps the vesicle, extending $30\ \mu$ anterior and $40\ \mu$ posterior to the anterior boundary of the vesicle. The connection with the ectodermal thickening mentioned above has also been lost by the disappearance of the anterior end of the preauditory placode, and $30\ \mu$ intervene now between the anterior end of the placode and the posterior limit of the ectodermal thickening, which extends $90\ \mu$ behind the actual contact of the endoderm of the gill pocket with the general ectoderm. The distance between the preauditory placode and the actual contact of the ectoderm and endoderm is, therefore, $120\ \mu$ in this stage.

b. Ectodermal gill thickening

The most conspicuous structure in the region between the auditory vesicle and the optic vesicle in early embryos is the ectodermal thickening where the endoderm of the hyoid gill pocket approaches the ectoderm. The significance of this structure is uncertain, as has been pointed out (Landacre '12), but its extreme size in this type and the fact that it occupies approximately the same level dorso-ventrally in which the sensory lines later appear makes it of importance in this discussion. As the endoderm of the hyoid gill pocket pushes out and approaches the epidermis, a thickening appears at, anterior to, and posterior to the point of contact (fig. 1). This ectodermal thickening lies at a slightly lower level than that occupied by the preauditory placode. In stages preceding the 88-hour stage, the ectodermal thickening extends caudad from a point $20\ \mu$ anterior to the contact of endoderm and ectoderm, from which point it curves upward and joins the anterior end of the preauditory placode. The ectodermal thickening is easily distinguished from the preauditory placode, since it is not marked by the peculiar cell arrangement so characteristic of the placode. The cells of the thickening are small in size, irregular in outline, and have deep-staining qualities. The relation of the ectodermal thickening to the preauditory placode at the 88-hour stage is shown in figure 1. In this stage the actual contact is $160\ \mu$ in length and the thickening is of less extent but more pronounced appearance than in earlier stages.

The changes in the thickening from the 88-hour stage (fig. 1) to the $120\frac{1}{2}$ hour stage (fig. 5). in which well developed sensory lines can be recognized, may be followed by a comparison of figures 1, 2, 3, 4 and 5. It will be seen that the ectodermal thickening continues to decrease in length. The actual contact of the endoderm and ectoderm shortens at the same time. In the 94-hour stage (fig. 2) the contact is $140\ \mu$ in length, and ends posteriorly just ventral to the region marked by the appearance of the primordium of the sensory lines.

In the 100-hour stage (fig. 3) the ectodermal thickening has changed little, but the actual contact is $100\ \mu$ in length. The

106-hour stage (fig. 4) shows an actual decrease in the extent of the thickening, and the contact of the endoderm and ectoderm is only 80 μ in length at this stage

The period between the 106-hour stage and the 120½-hour stage is characterized by a marked decrease in the extent of the ectodermal thickening. The structure becomes rhomboidal in outline and is elongated in the antero-posterior axis, which makes an angle of twenty-eight degrees with the roof of the pharynx. The character of the thickening at this stage is shown in figure 5. The actual contact of the ectoderm and endoderm is 50 μ in length. The sensory lines are well-developed in the 120½-hour stage and lie somewhat dorsal to the ectodermal thickening.

c. Epibranchial placode

The third structure which lies in the region between the auditory vesicle and optic vesicle is the epibranchial placode. It can be recognized easily by the small size and dark-staining qualities of its cells. The epibranchial placode can first be identified about the time the preauditory placode disappears and during the maximum development of the ectodermal thickening or contact in the region where the endoderm of the hyoid gill pocket approaches the epidermis.

In the 94-hour stage (fig. 2), the ectodermal thickening at the posterior end of the contact of the hyoid gill pocket and ectoderm extends farther mesially than in sections anterior to that point, and an examination of later stages shows this to be the first appearance of the epibranchial placode. In the 106-hour stage (fig. 4) the epibranchial placode can be positively identified, and from this stage the further development of the structure can be traced until it finally is detached from the ectoderm and joins the general visceral portion of the VII ganglion. In the 106-hour stage, the epibranchial placode consists of a mass of cells projecting mesially in the region of the ectoderm formerly occupied by the posterior extension of the ectodermal thickening.

The 112-hour stage exhibits little change in the epibranchial placode, and aside from a gradual increase in size and the darker blue stain taken by the cells, the placode shows little change

to the time of detachment. Between the 137-hour stage and the 154-hour stage, the placode becomes completely detached and its later history (see Landacre '12) will not be followed here, since lateral lines can be recognized previous to this time. There is, however, little likelihood of confusing the two structures, even though they be close together, on account of the differences of histological structure.

d. The posterior extension of the epibranchial placode

The fourth of the structures, whose presence complicates the study of the region anterior to the auditory vesicle, is the ectodermal thickening which extends caudad from the epibranchial placode, and which we shall call 'the posterior extension of the epibranchial placode,' following the usage of that term by Landacre ('12).

As has been previously pointed out, the epibranchial placode makes its appearance in the 94-hour stage, at a time when the posterior portion of the preauditory placode still persists from a point $30\ \mu$ in front of the anterior boundary of the auditory vesicle, back to a point $40\ \mu$ posterior to the anterior boundary of the auditory vesicle. In the 94-hour stage there is an area of $70\ \mu$ intervening between the posterior limit of the cells of the epibranchial placode and the radially arranged cells of the preauditory placode. The region between the placodes presents the appearance of normal ectoderm.

In the 100-hour stage, a structure has appeared in the region from which the preauditory placode has disappeared. This is the posterior extension of the epibranchial placode. There is no difficulty in distinguishing between the preauditory placode and the posterior extension of the epibranchial placode (fig. 8). In the former, the cells are columnar, with a radial arrangement, while in the latter the cells are small in size, irregular in outline, and show no definite arrangement.

During the period between the 88-hour stage and the 94-hour stage, as mentioned above, the anterior end of the preauditory placode degenerates. In the 88-hour stage the radial arrangement of the cells of the placode may be recognized for a distance

of 100 μ in front of the anterior boundary of the auditory vesicle, while in the next stage (94 hours) the preauditory placode extends only 30 μ in front of the anterior limit of the vesicle and at 100 hours it is no longer present.

On the other hand, the posterior extension of the epibranchial placode can first be recognized in the 100-hour stage. From the fact that the posterior extension of the epibranchial placode is continuous with the epibranchial placode, while the preauditory placode has already disappeared and that it exhibits none of the histological characters of the preauditory placode, and that its growth backwards follows the receding of the preauditory placode after a lapse of several hours, we may assume that there was no genetic relation between the preauditory placode and the posterior extension of the epibranchial placode. Previous to the stage when the posterior extension of the epibranchial placode is recognizable (fig. 3), the primordium of the anterior sensory lines is already formed, anterior to and dorsal to the epibranchial placode, as shown in figures 2 and 3.

e. The primordia of supra-orbital and infra-orbital sensory lines

In the 94-hour stage (fig. 2) an ectodermal thickening appears in the region just dorsal and anterior to the epibranchial placode. Examination of later stages show this structure to be the primordium of the sensory lines upon which the lateral line organs of the supra-orbital and sub-orbital lines will later be formed.

As has been previously pointed out, the anterior end of the preauditory placode degenerates, so that, in the 94-hour stage (fig. 2), the radial arrangement of its cells can be recognized for 30 μ only in front of the anterior limit of the auditory vesicle. Between the anterior end of the preauditory placode and the posterior limit of the epibranchial placode, which can first be recognized in this stage, a distance of 70 μ intervenes. Since the preauditory placode lies at a level slightly dorsal to that occupied by the epibranchial placode, and in addition certain histological differences may be noted, the two structures are easily distinguished.

At a time, then, when the preauditory placode has shortened so that $70\ \mu$ intervene between its anterior limit and the posterior limit of the epibranchial placode, the first primordium of the sensory lines appears (fig. 2, 94 hours). Its cells do not exhibit a pronounced radial arrangement in this stage, but the cells are elongated, and the majority of the cell nuclei are found in the mesial ends of the cells.

The position occupied by the primordium of the sensory lines in the 94-hour stage is dorsal and anterior to the position occupied by the preauditory placode in the preceding stage. In stages prior to the 94-hour stage (fig. 1), the preauditory placode showed a sharp downward flexure at the point where it joined the ectodermal thickening which extended caudad from the region where the endoderm of the hyoid gill pocket approached the epidermis.

Examination of a number of individuals at the 94-hour stage, exhibiting variations in the degree of development, fails to show any evidence for a genetic relation between the preauditory placode and the primordium of the sensory lines. That there is no such relation seems all the more probable, since the anterior end of the placode at no stage occupied the region marked later by the appearance of the primordium of the sensory lines, but extended forward with a downward flexure in the region just posterior to the epibranchial placode. This flexure is shown in figure 1.

The 100-hour stage (fig. 3) shows a further development of the primordium of the sensory lines, in that the radial arrangement of the cells is more pronounced, so that the structure is well differentiated by histological characters from the ectodermal hyoid thickening which lies at a slightly lower level. The sensory line primordium is $140\ \mu$ in length in the 100-hour stage, and lies parallel with the roof of the pharynx.

The 106-hour stage (figs 4 and 21) exhibits little change over the preceding stage as regards the development of the primordium of the supra-orbital sensory lines. In this stage, however, the first trace of the primordium of the mandibular line is found. It lies ventral to the middle portion of the hyoid ectodermic thickening and completely detached from it. In this case there

can be no question of the distinct origin of this line entirely separate from the preauditory placode, since its first appearance is entirely distinct, not only from the preauditory placode, but also from the three structures associated with the formation of the gill slit.

The period between the 106-hour stage and the $120\frac{1}{2}$ hour stage is marked by the appearance of definite supra-orbital and infra-orbital lines. (figs. 13 and 15). The anterior end of the primordium bifurcates and from the bifurcated ends the two sensory lines originate. The mode of origin of the supra-orbital and infra-orbital lines is shown in figures 9, 10, 11 and 12, which are drawn from an embryo of 137 hours, in which the development was below normal, so that it may be taken to represent conditions prior to the previous stage ($120\frac{1}{2}$ hours). It will be seen that the supra-orbital and infra-orbital lines appear to be cephalad continuations of the common supra-branchial primordium of the lateral lines.

After the appearance of the supra-orbital and infra-orbital lines, the primordium degenerates posterior to the point of origin of the lines, and in later stages, we could find no evidence of an anastomosis between the supra-orbital and sub-orbital lines.

In the $120\frac{1}{2}$ -hour stage (fig. 5), the supra-orbital line is $310\ \mu$ in length. It begins at a point $90\ \mu$ in front of the anterior limit of the auditory vesicle and directly dorsal to the median point in the line of contact between ectoderm and endoderm in the region of the hyoid gill pocket. From this point it may be traced cephalad and dorsad to a point $50\ \mu$ behind the posterior boundary of the optic vesicle and at the level of the dorsal limit of the flexure of the brain-floor immediately below the mesencephalon.

The infra-orbital line begins at a point $30\ \mu$ in front of the posterior limit of the supra-orbital line at a slightly lower level and distinct from it. From this point it may be traced forward for $100\ \mu$, then cephalad and ventrad until it ends $30\ \mu$ behind the posterior boundary of the optic vesicle and at the level of the hypophysis. The extent of the lines and their relation to surrounding structures is shown in figure 5.

The 154-hour stage (fig. 6), shows a marked development in the sensory ridges. A description of the sensory lines in this stage has already been given under the the general description of the 10-mm. embryo (p. 583), to which the reader is referred.

f. The primordium of the hyo-mandibular line

Owing to the acute angle at which the transverse sections cut the hyo-mandibular line, the radial arrangement of the cells is not so pronounced as in the supra-orbital and infra-orbital lines. and for that reason the hyo-mandibular line is less easily recognized than the other lines anterior to the auditory vesicle.

In the 106-hour stage (fig. 4) a mass of cells, scarcely differentiated from the contiguous ectoderm, may be observed at the level of the roof of the pharynx, and directly beneath the line of contact of the ectoderm and endoderm in the region of the hyoid gill pocket. The structure can be identified in three sections, since its cells project farther mesially than do the ectodermal cells anterior and posterior to this region. An examination of later stages show this to be the first appearance of the hyo-mandibular line. In the 120½-hour stage (figs. 5 and 20), the hyo-mandibular line has grown cephalad and sharply ventrad, so that it may be traced from the region immediately below the hyoid contact and at the level of the roof of the pharynx until it is lost in the thickened ectoderm in the lateral angle where the embryo joins the yolk sac.

The position in which the primordium of the mandibular line arises is so far ventral to the primordium of the supra- and infra-orbital lines that it furnishes strong evidence of its independent origin; furthermore, it is separated from the dorsal primordium of the lateral line by the thickening of the epidermis including the hyoid thickening, the epibranchial placode and the posterior extension of the epibranchial placode (fig. 4). Even if intermediate series should show it to be farther dorsal than indicated in figure 4, the presence of these structures is a barrier to conceiving it as having a common origin with the dorsal primordia or the preauditory placode.

THE POSTAUDITORY REGION

Since there is no posterior extension of the auditory vesicle in *Lepidosteus* from which the lateral line primordia could be derived, the problem of the relation of the auditory vesicle to this primordium would seem at first glance simple of solution. Such is not the case. The existence in the preauditory region, of four distinct structures, three of which are concerned in the formation of the gill slit and the epibranchial placode, and their relation to the lateral line primordia and especially the fact that they are so situated that they seem to furnish a continuity in structure between the vesicle and the lines emphasize the necessity of a careful study of the postauditory region, even though the postauditory placode is absent. It is certainly present in some types and in a position corresponding to the preauditory placode. Whether it is present and atrophies, as in *Ameiurus*, or is absent, as in *Lepidosteus*, there is the same need for a careful study of all the structures that might be mistaken for it or might seem to render continuous the vesicle and the primordia of the lines.

In the postauditory region there are present all of the structures associated with the gill slit that are found anterior to the ear, namely: (a) the thickenings of the epidermis at the points where the endoderm of each gill pocket joins the ectoderm; these usually appear previous to the opening of the gill slit; (b) the epibranchial placode of each gill; these are situated at the posterior end of the ectodermic thickening (a); (c) The later thickening of the ectoderm arising behind the area of contact of endodermic gill pocket and ectoderm, these appearing as a posterior extension of the gill thickening. This last thickening persists, however after the gill slit has opened and after the epibranchial placode has become detached from its appropriate gill and has joined the visceral ganglia of the IX and X nerves. (d) In addition to all these structures there is present in the postauditory region, especially in stages preceding the contact of the endodermic gill pockets with the ectoderm, a thickening sometimes quite pronounced, at the point where the more or less vertical wall of the body turns laterally to extend over the yolk sac.

At this point the ectoderm is materially thickened especially preceding the appearance of the thickening of the epidermis at the actual point of contact of ectoderm and endoderm of the gill pocket. In fact, the gill pocket thickening at its posterior end is usually continuous with the more ventral thickening. Whether this primitive thickening at the point where the vertical body wall turns at nearly right angles to join the wall of the yolk sac is to be thought of as a precursor of the gill pocket thickening or as a strengthened area in the wall, the writers are unprepared to say. It is of special interest at this time on account of the ease with which it might be taken for a lateral line primordium. In fact, both writers did mistake it for a postauditory placode, and at a later stage for the primordium of the body line. It was only after the true body line primordium had been followed to its earliest stages, and the earliest stages of the gill pocket thickenings of the epidermis had been followed, that it was discovered that it had nothing whatever to do with the lateral lines but was intimately associated with the gill slit thickenings, if not the actual precursor of them. In view of these facts, a rather detailed description of all these structures will be given, up to and including the appearance of the primordium of the postauditory or body sensory line.

Stage XIII. The description of the postauditory thickenings in the ectoderm will begin with Stage XIII. At this time the posterior end of the auditory vesicle ends in a rounded knob whose posterior extremity lies nearer the neural canal than it does to the ectoderm. There is no thickening in the epidermis posterior to the hinder end of the vesicle and on a level with it, and there has been none in the stages preceding the one under discussion. There is a thickening lying at a lower level and related in position to the gill pocket in this stage, but it could hardly be mistaken as to its relations.

The pharyngeal pocket of the first true gill is in contact with the ectoderm throughout twenty-one sections (of $10\ \mu$ each) but has not as yet opened to the exterior. Almost the whole area of contact lies anterior to the anterior limit of the auditory vesicle. There is an overlapping of approximately five sections. This

relation cannot be stated more definitely on account of the difficulty of determining the exact anterior end of the vesicle owing to the fact that it passes forward at this stage gradually into the preauditory placode. In the last five sections of the area of contact of the pharyngeal endoderm there appears a thickening of the epidermis at the point of contact. Anterior to this thickening the ectoderm is thin (fig. 23). The appearance of the thickening is shown in figure 24. It lies ventro-laterally from the auditory vesicle and the epidermis is thinner between the two structures than it is at the point of contact of the gill pocket with the ectoderm. Figure 24 is taken one section anterior to the point where the gill pocket is no longer in contact with the ectoderm. This thickening extends $100\ \mu$ back of this point, so that of its total extent one-third is co-extensive with the posterior portion of the area of contact of the pharyngeal pocket of the first true gill and two-thirds lies posterior to that contact. Figure 25 shows it $50\ \mu$ further back than figure 24; from this point it diminishes gradually and its posterior end can be located with difficulty. It certainly does not reach at this stage the level of the posterior end of the auditory vesicle (fig. 26), but does reach approximately the point at which the auditory vesicle becomes detached from the epidermis.

This thickening can be characterized briefly as an ectodermal thickening lying ventro-laterally from the auditory vesicle, the anterior third of which is co-extensive with the posterior end of the gill pocket contact, the posterior two-thirds extending backward in the ectoderm behind the gill pocket contact, and further the whole length of the thickening is approximately co-extensive with the area over which the auditory vesicle is still in contact with the ectoderm at this stage. Its detachment from the auditory vesicle, its more ventro-lateral position and its evident connection with the gill pocket preclude referring it in any way to the ear or lateral line system. Its later history shows it to be the primordium out of which the epibranchial placode forms as clearly as its early history shows it to be due in some measure to the existence of the forming gill slit.

Stage XIV. Stage XIV, 6 hours older (fig. 27) than the preceding, shows no striking changes, in the auditory vesicle and preauditory placode, which are still attached to the ectoderm throughout most of their extent, although the posterior end of the vesicle is detached through a slightly greater extent than in the preceding series. The most evident changes are in the ectodermal thickening connected with the gill pocket. The detail of this thickening is shown in figure 7 which is taken anterior to that of figure 27, and lies just behind the area of contact of the first true gill.

This thickening has extended somewhat further backward and now reaches nearer to the posterior end of the auditory vesicle which here, as in the preceding series, lies nearer to the medulla oblongata than to the ectoderm. The posterior portion of the thickening extending behind the area of contact is in this series somewhat longer dorso-ventrally, so that it extends down from its position in the preceding series to the angle (fig. 27) where the lateral body wall joins the nearly horizontal wall which extends over the yolk sac. In a later stage the first gill pocket thickening will separate from a second one associated with the second true gill and the first will be found to end blindly in the ectoderm as in the case of the hyoid gill thickening. There is, however, only a slight indication of a division into two in this series.

Stage XV. In this stage the posterior half of the auditory vesicle is detached from the ectoderm. The pharyngeal pocket of the second true gill has not reached the ectoderm. When it does reach the ectoderm its anterior end would lie directly under the area of contact of the preceding gill, and the same condition is true of the remaining gills, so that their ultimate arrangement is like the shingles on a roof, the anterior end of each endodermic pharyngeal pocket lying ventral to the preceding area of contact. Posterior to the area of contact in each pharyngeal pocket there is the thickening of the ectoderm as in the case of the hyoid gill.

While the pharyngeal pocket of the second true gill has not reached the ectoderm, the ectodermic thickening over the area of future contact is present and slightly separated by a groove on

the mesial surface from the dorsal thickening of the first true gill (fig. 28). This separation is not complete as yet but becomes so later, proceeding from in front backward; at the posterior end of the more dorsal thickening the two are confluent. The ventral thickening, it will be noticed from figure 28, occupies the lateral body angle formed by the nearly vertical body wall and horizontal body wall. It is an interesting fact that this more ventral thickening becomes partially separated and is certainly in existence before the endodermic gill pocket reaches the ectoderm.

This more ventral ectodermic thickening, which for convenience can be called the angular thickening, since it lies in the angle at the side of the body, can be best interpreted, at least over the area of contact, as the forerunner of the ectodermic portion of the gill slit. This is very slight in an open slit but still is present. It is no more remarkable that ectoderm should undergo changes preceding the contact than that the endoderm should produce a well defined evagination before it comes into contact with the ectoderm. The exact position of the more ventral portion of this common thickening before it separates from the more dorsal is apparently due solely to the position of the future gill slit. This, as a glance at figures 28 to 34 will show, is quite low on the side of the body and near to the yolk. The term angular thickening as applied to this region of thickened ectoderm may prove to be quite superfluous. If it represents only an early stage in the appearance of each ectoderm gill thickening, it is not needed. If, however, it should be concerned in strengthening the side wall of the body at the angle, its use would have more justification. At any rate, it is a convenient means of designating the common thickening out of which the true gill thickenings form and which precedes the contact in appearance.

Stage XVI. In this stage the auditory vesicle is detached from the ectoderm except at its extreme anterior end. The endodermic pocket of the second true gill touches the ectoderm over an area corresponding to the anterior end of the more ventral thickening of the preceding stage (fig. 29). The posterior end of the more dorsal thickening extends back over the anterior end of the more ventral (fig. 1), in fact beyond the

region of contact of the second gill pocket, and the two merge into a rather long thickening dorso-ventrally but separated by a marked groove.

The two thickenings as shown in figure 1 do not represent exactly the same relation in respect to the contact with their respective gill pockets. The dorsal thickening represents chiefly the extension of the thickening posterior to the detachment of ectoderm from the endoderm pocket, while the more ventral includes at its anterior end also the area of contact between the ectoderm and the endoderm. The anterior portion of this thickening will later be included in the gill slit and only the posterior will remain as thickened cord in the ectoderm. Since the object is to trace all thickenings that could possibly be taken for lateral sensory lines, this figure is given just as it appears on the slides.

Stage XVII. In stage XVII the auditory vesicle is completely detached from the ectoderm. The more dorsal thickening of the preceding stages (the first gill thickening) is entirely separated even at the posterior end from the more ventral (figs. 2 and 14 for detail) which is associated with the second true gill. The greater portion of the dorsal thickening, a length of nine sections out of a total of thirteen, lies behind the area of contact of the first true gill and its cells take a dark stain, a peculiarity which precedes the formation of the epibranchial placode previous to the detachment of the epibranchial or special visceral ganglion. However, not all of this as it is found in this series is concerned in the formation of the epibranchial ganglion, only the anterior end being so used, the posterior end disappearing.

The more ventral thickening (fig. 2) is longer than in the preceding stage (fig. 1). It is broader at its anterior end than at the posterior end and there are indications of a division into two parts (fig. 30), although this is not so pronounced as in the next stage. So far as a third ventral thickening can be distinguished, it is the thickening at the angle of the body as in the early stage of the second thickening for the second true gill. As in the preceding reconstruction (fig. 1) the more ventral thickening of figure 2 consists principally of contact area where the second true gill touches the ectoderm. Behind the area of contact of the

second true gill, this thickening becomes one with the thickening in the lateral angle of the body, so that it is not possible to determine exactly how much of it lies behind the area of contact.

Stage XVIII. Stage XVIII is characterized by a marked degree of separation of the thickening at the contact of the second true gill into two parts at its anterior end (fig. 3). Figure 18 shows the detail in the middle of the thickening extending behind the first gill contact. The portion of the second thickening lying above the gill slit represents partly the area of contact but mostly a posterior extension behind the area of contact of the second true gill.

The part lying below the split represents the thickening at the angle of the body. This will be chiefly contact of the third gill pocket in the next series. The two thickenings are continuous, as indicated in figure 3, throughout the posterior three fourths. Figure 31 shows the appearance of the two thickenings just behind the area of contact of the second true gill. The whole ventral half of the second thickening lies at the angle where the ventral lateral body wall becomes horizontal as it passes out over the yolk. The posterior end of this combined dorsal and ventral thickening extends backward into the region of contact of the third true gill.

Stage XIX. Stage XIX (fig. 4) shows striking changes in the further differentiation of gill thickenings, as well as in the appearance of the first trace of the postauditory lateral line primordia.

In the region of the second true gill (fig. 4) the thickening which in the preceding stage was confluent at its posterior end with a more ventral thickening is now quite distinct and ends free behind. The second thickening plotted in figure 4 is mainly a posterior extension behind the area of contact, while the third thickening represents mainly the area of contact of the third true gill and at its posterior end drops to a lower level than at its anterior end, and occupies again the angle at the side of the body. It also extends well back into the area of contact of the fourth true gill.

The primordium of the lateral line (fig. 4, *Po.L.L.*) is only slightly developed in this series, but it is quite pronounced in the next series plotted and occupies the same position as in the series under discussion, so that while it is indefinite there can be no doubt as to its identity. This primordium is not the forerunner of the main body line which appears more posteriorly in the next series plotted (Stage XXI), but is the primordium of the group of lateral line organs supplied by the ramus supratemporalis IX arising from the lateralis IX ganglion.

The position of the lateral line primordium in the longitudinal axis is coextensive with the posterior fourth of the auditory vesicle. Dorso-ventrally it lies at the level of the lower border of the auditory vesicle. It is most distinct anterior to the thickening of the first true gill, but seems to extend back over that thickening, as indicated in figure 4. It is quite detached from that thickening and lies at a higher level.

The appearance of this primordium quite distinct from both gill thickenings and auditory vesicle is in harmony with conditions in the preauditory region as described in the first section of this paper. The auditory vesicle at its posterior end has been detached from the epidermis for more than thirty-two hours and during all that time there has been no trace of a primordium of the lateral line organs, and there seems to be no doubt that the primordium first arises as a localized differentiation in the ectoderm quite separate from any connection with the auditory vesicle. Its relation to the origin of the lateralis IX ganglion, as well as the relation of the main line to the lateralis X ganglion, is not so clear. No trace of either of these ganglia can be positively identified in this stage.

Stage XX. Stage XX, which lies in point of time between the last stage and Stage XXI shows no advance on stage XIX.

Stage XXI. Stage XXI (fig. 5) is characterized by the presence of four thickenings associated with the gills and by the presence of two lateral line primordia in addition to the one mentioned in Stage XIX.

The two additional primordia found in this stage will be designated the second and third postauditory and are associated with

the lateralis ganglion of X, just as the more anterior is associated with the lateralis ganglion of IX.

The ectodermic gill thickenings, as mentioned above, are four in number at this stage, three of them lying behind the first three true gills and the fourth lying chiefly in the area of contact of the fourth gill. Since the lateral line primordia are present, it will not be necessary to follow these thickenings further than to mention the fact that the fourth behaves just as the other three, that is, forms an extension behind the fourth true gill and later a fifth is formed in a manner quite similar to the other four. It may be of interest here to recall the fact shown by Landacre ('12) that the epibranchial placodes of the five gills associated with the ninth and tenth nerves can be positively identified first at the following ages; the epibranchial placode of the first gill in Stage XXII; of the second and third gills in Stage XXVI.

Of the three lateral line primordia present in this series the more anterior occupies relatively the same position as in the preceding series with the exception that, owing apparently to a change in the shape of the body, it is situated somewhat more ventrally with reference to the auditory vesicle. It has increased in size and lies as before at the level of the posterior border of the auditory vesicle. The posterior end of the lateral line primordium extends slightly beyond the posterior end of the auditory vesicle (fig. 5). It lies approximately parallel to the thickening of the first true gill and is about one-half as long as that thickening. Its anterior end lies at the posterior base of the opercular fold which is first distinguishable in this series, and it extends from this point backwards over the thickening of the first true gill and at a considerably higher level (fig. 32). At its posterior end it comes closely into contact with the lateralis ganglion of the IX nerve.

The condition here, as well as the relation of the lateralis X ganglion to the lateral line primordium, suggests strongly that these ganglia may be derived from the same primordia that give rise to the lateral line organs in these regions respectively. However, our series are not taken at sufficiently close intervals to

settle this interesting point. Stages XXI and XXII are unfortunately the only stages in the series up to Stage XXX that are more than seven hours apart and even this interval probably would have been too long to settle it.

The most posterior primordium of the postauditory lateral line, the third in position, lies posterior and, at its anterior end at least, dorsal to the third gill thickening (fig. 5). Its anterior end begins at the transverse level of the posterior end of the third gill thickening, and from this point it extends back entirely beyond and dorsal to the fourth gill thickening. Behind the fourth gill thickening it drops to a lower level but not as low as the angular thickening on the side of the body. The angular thickening in this stage does not extend posterior to the posterior end of the future fifth gill thickening, so that the third lateral line primordium extends posterior to the gill region.

The relation of this lateral line primordium to the lateralis X ganglion is interesting. The lateral line ganglion of the X occupies $200\ \mu$. It is largest in its middle region and at its posterior end comes quite close to the ectoderm (fig. 34). The lateral line primordium is $300\ \mu$ in length and its anterior end overlaps the first five sections ($50\ \mu$) of the lateralis X ganglion where it comes quite close to the ectoderm. In fact, the two structures are almost in contact, suggesting strongly, as was indicated in discussing the more anterior lateral line primordium, that the ganglion is derived by splitting off from the lateral line placode. From the posterior end of the ganglion the lateralis X nerve runs back only $50\ \mu$ beyond the posterior end of the ganglion, leaving $200\ \mu$ of the lateral line primordium with no accompanying nerve, a fact which furnishes rather striking evidence that the lateral line nerve is not split off from the ectoderm but grows out from the ganglion, as other nerves do.

Histologically the posterior lateral line primordium shows less resemblance in these earlier stages to the lateral line organs than do those in the hyoid region. This resemblance occurs a little later and its absence at this stage may possibly be accounted for, by the sudden appearance and rapid growth of the structure. The later history of this primordium as follows: The posterior

end grows rapidly back along the side of the body and in Stage XXIII is more than 124 sections long.

In this same series there has appeared another primordium, the second in position, and anterior to the third or between the first and third primordia, as shown in figure 5, and near the anterior end of the lateralis X ganglion, whose lateral line organs are supplied by the ramus supratemporalis X (figs. 5 and 33). In Stage XXIV, where the nerve is sufficiently well developed to trace it into the ectoderm in the vicinity of this third thickening, the thickening lies with its anterior end over the second epibranchial thickening and extends from this point back beyond the epibranchial thickening.

In Stage XXVI, plotted by Landacre ('12), the lateral line ganglion on X is shown as a single ganglion, quite long, from the anterior end of which the ramus supratemporalis X arises. This plot represents accurately the conditions at this stage. But in earlier series XXIII and XXIV, there seems to be a second small lateralis ganglion at the anterior end of lateralis X from which the ramus supratemporalis X arises. The separation between the two is not absolutely sharp but in view of the findings in the frog (Landacre and McClellan '13) and in the urodeles, as shown in an unpublished paper by Kostir, it is extremely probable that there are two lateral line ganglia on X which later, as in the Anura and urodeles fuse into one common ganglion.

In addition to the organs of the main lateral line innervated by the ramus supratemporalis of X and those innervated by the chief lateralis X ganglion, there are in series XXVII additional organs lying above the main lateral line ganglion innervated by that ganglion.

As to the relation of these three primordia to each other, there is no evidence from our series, although they are not as close together as could be desired, that all three arise from a common primordium. They appear to arise as three separate primordia. It is practically certain that the first lateral line primordium supplied by the ramus supratemporalis IX is not connected genetically with those lying posterior to it. Its extremely small size in Stage XIX and evident increase in size in Stage XX,

where it is separated by unmodified ectoderm from the second primordium, preclude such an idea. Even in the case of the last two, there is a sufficient amount of unmodified ectoderm between them in the first series in which they appear to make it extremely improbable that they were ever continuous, although it is a possibility.

SUMMARY AND DISCUSSION

The preauditory region

1. The lateral line primordia arise in *Lepidosteus* quite distinct from the auditory vesicle, but so situated with reference to other thickenings associated with the gills, both the hyoid and the true gills, that all these structures must be followed in a close series to insure their differentiation.

2. In the preauditory region there are the following structures to be differentiated: (a) The preauditory placode or anterior extension of the auditory vesicle; (b) The thickening of the ectoderm at the point where the endodermic gill pocket of the hyoid gill comes into contact with the ectoderm; (c) The posterior extension of *b* which grows back of the area of contact of the endodermic pocket with the ectoderm. In this posterior extension develops the epibranchial placode; (d) The epibranchial placode which appears after *a* and *b* and before *c* and on account of its size and character is not a particular source of difficulty; (e) The lateral line primordia; these appear after *a* and *b* and consist of a common primordium for the supra- and infra-orbital lines and one for the mandibular line.

3. The preauditory placode appears approximately at the same time as *b*, the ectoderm gill thickening. It is the anterior extension of the auditory vesicle, resembling it in histological structure but is smaller in dorso-ventral diameter. Before a cavity appears in the vesicle it is difficult to locate the anterior end of the vesicle on account of a gradual transition of the vesicle into the placode. In the early stages the anterior end of the preauditory placode extends forward to the hyoid region and abuts

against the posterior region of the ectodermic gill thickening. The two ectodermic thickenings are, however, quite different histologically in that the preauditory placode resembles the auditory vesicle. The preauditory placode next degenerates from before backwards and finally disappears about the time the first trace of the lateral line primordia can be detected. The last trace of the preauditory placode was observed at the anterior end of the auditory vesicle and the first trace of the lateral line primordia appears just over the middle of the hyoid gill thickening having a considerable area of unmodified epithelium between them. When followed in a close series there is no indication of a connection between the preauditory placode and the primordium of the sensory lines.

4. The hyoid ectodermal thickening appears simultaneously with or previous to the contact of the hyoid gill with the ectoderm. During the time of contact of the gill pocket with the ectoderm the thickening extends the whole length of the contact and also projects anterior to the area of contact. This anterior extension however varies little in extent during the whole existence of the thickening and seems to have no special significance; at least, it is not a source of confusion.

Of more importance is the existence of the posterior extension behind the area of contact. This is present in the very early stages of the thickening and at its posterior end abuts against the anterior end of the preauditory placode, so that if it were not for the difference in histological structure one could quite easily read the preauditory placode forward through the whole extent of the contact of the hyoid gill with the ectoderm. That this is not the case is shown by the histological structure as mentioned above, as well as by the different fates of the two structures. The behavior of similar thickenings in the case of the five true gills renders this conclusion practically certain.

The posterior extension of the hyoid gill thickening persists throughout the whole existence of that thickening, which lasts up until well defined lateral line primordia are present in both preauditory and postauditory regions. It disappears finally with the last remnant of the gill thickening.

Its importance can hardly be overestimated on account of its position. It is so located with reference to the preauditory placode and to the primordia of the lateral lines that unless it is followed with extreme care it seems to render these two structures continuous in the ectoderm and would naturally lead to the theory of the continuity of auditory vesicles and lateral line primordia. Whatever theory is substituted for the theory of continuity of auditory vesicles and lateral lines, in the opinion of the writers the theory of continuity must be abandoned. The most reasonable theory in our opinion is that the sensory areas of the auditory vesicle and the lateral line organs are homologous structures with a more or less longitudinal distribution on the body. The auditory sensory areas and the lateral line sensory areas are on a par and the auditory vesicle is in no sense the parent of the lateral line primordia.

5. At an early stage in the history of the posterior extension of the gill thickening the epibranchial placode can be located. Since, however, its history has been followed in detail in an earlier paper (Landacre '12) it will not be described here. In its earlier stages and before detachment of the epibranchial ganglion it forms an intermediate mass in the posterior extension of the gill thickening.

6. The first primordium of the lateral lines that appears is the common primordium of the supra- and the infraorbital lines. This appears in the same series in which the last trace of the preauditory placode can be found. The preauditory placode previous to the appearance of the lateral line primordium has been undergoing a process of degeneration as described above. When the lateral line primordium first appears it occupies a position exactly dorsal to the epibranchial placode and in contact with the placode on its ventral surface, but distinguishable from it by its histological character, resembling the preauditory placode in the radial arrangement of its cells, while the epibranchial placode and the whole gill thickening, in fact, have irregularly arranged cells. It is separated from the remnant of the preauditory placode, however, by an area of unmodified ectoderm equal in extent to the total length of the lateral line primordium itself.

After its appearance it grows rapidly forward and seems to split into the supra-orbital and sub-orbital lines in which later the lateral line organs appear. The lines are quite distinct from each other at their posterior ends soon after their appearance.

The primordium of the mandibular line appears later than that of the supra- and infra-orbital lines. It lies ventral to the hyoid thickening from which it is completely detached. Its position in a dorso-ventral diameter is on a level with the anterior end of the hyoid thickening. Its late appearance and complete detachment from other thickenings in the hyoid region leave no doubt as to its distinct origin.

The postauditory region

1. Under the term 'postauditory region' is included all that area whose lateral line organs are supplied by the IX and X nerves. The area supplied by the IX nerve lies at the level of the ear and is not strictly postauditory in position, although supplied by a postauditory nerve. In the postauditory region there is no postauditory placode or posterior extension of the auditory vesicle and the problem as to the origin of the postauditory lateral lines from the vesicle is easily disposed of. There remains only the problems as to what structures are so situated as to be mistaken for lateral line primordia and the exact time and place of appearance of the lateral line primordia.

2. In the postauditory region occur the same structures with the exception of the auditory placode that were found in the preauditory region. These structures are more numerous on account of the presence of five functional gills; but on the whole are easier of interpretation on account of the fact that the gills become functional, whereas the hyoid gill behaves as if it were going to open but later the endodermic pocket withdraws from the ectoderm leaving the epibranchial placode to furnish the only permanent contribution to structures in the head. The postauditory region is further rendered easier of interpretation on account of the repetition in each gill of similar structures, thus furnishing a basis for comparison of the several gills with each other and with the hyoid gill, and lastly by the absence of a postauditory placode.

3. The structures lying on a level with the auditory vesicle and posterior to it are: (a) A common thickening of the ectoderm at the side of the body where the more or less vertical body wall becomes horizontal when it turns laterally to spread out over the yolk. This is a common primordium out of which the various gill thickenings develop and may have no significance aside from the development of these thickenings. (b) The ectodermic thickening at the point of contact of each endodermic gill pocket with the ectoderm. (c) The posterior extensions of these thickenings behind the area of contact of each gill. (d) The epibranchial placodes developed as mesial extensions of each thickening at the dorsal and posterior border of each gill slit. (e) The primordia of the lateral line, of which there are three. In the first of these develop lateral line organs supplied by the ramus supratemporalis IX; in the second organs supplied by the ramus supratemporalis X; in the third organs supplied by the main lateral line nerve of X.

4. The angular thickening, as it has been designated in the body of the paper, can be best described in connection with the gill thickenings. This is the first thickening of the ectoderm to appear in the postauditory region and is a longitudinal thickening of the ectoderm over the area in which endodermic pocket of the first true gill will come into contact with the ectoderm. This thickening appears, at least in the case of the last four gills and probably in the case of the first, before a contact is formed. After a contact is formed between the endoderm and the ectoderm in the case of the first gill the anterior end of the first thickening is split into two portions, the more dorsal of which represents the area of contact of the first true gill, and the more ventral of which represents the area of contact of the second true gill and lies at the extreme lower border of the lateral wall of the body. Posterior to this area of contact of the first true gill, both thickenings are continuous and much broader dorso-ventrally than either of the separated portions at the anterior end.

The endodermic pocket of the second true gill comes into contact with the more ventral of the two thickenings mentioned above. At the posterior end of this second thickening it becomes

broad and is again continuous with the more ventrally located angular thickening. Each contact of the endoderm with the ectoderm is placed diagonally on the body with the anterior end of the contact lower than the posterior end after the second contact is formed. The first becomes free at the posterior end, where it extends back of the area of contact.

This process is repeated in the case of the remaining gills. Each gill thickening is continuous at first at its posterior end with the angular thickening, later becomes separated near its anterior end from the more ventral angular thickening and finally also at its posterior end, after which it remains some time as a distinct thickening, at least until after the formation of the epibranchial ganglion of each gill, and then later disappears.

The gill thickenings are placed diagonally in the body with the anterior end lower than the posterior, so that their general relation to each other is like that of the shingles on a roof. This relation makes clear the splitting of each thickening at its anterior end before the thickenings of two successive gills become detached at their posterior ends.

The gill thickenings are to be looked upon as the ectodermic portions of the gill which is formed before the contact between ectoderm and endoderm is made, just as the endodermic gill pocket is in process of formation before the contact stage is reached. The import of the angular thickening is less easy to understand. It may have no significance further than its relation to the gill thickenings, or it may have something to do with strengthening the body at the point where the vertical lateral body wall turns and becomes horizontal as it spreads over the yolk. It seems not to be present after the yolk is further absorbed and the gills have formed, and its appearance is closely associated with the appearance of the gill slits. The position and relation of both the angular and gill thickening to surrounding structures are such that unless they are followed closely they might be mistaken for the postauditory placode or the primordia of lateral lines.

5. The posterior extensions of the gill thickenings, as well as the epibranchial placode, are closely associated with gill thicken-

ings themselves. As each gill thickening becomes detached from the more ventral angular thickening, its posterior end extends back of the area of contact of the endodermic gill pocket with the ectoderm and persists after the gill opens. This posterior extension becomes detached from the angular thickening after the anterior end is free from the same thickening, as mentioned above. At the anterior end of this extension or at the posterior border of the gill slit the epibranchial ganglion is detached from the ectoderm, after which the posterior extension gradually disappears. The significance of the posterior extension of the gill thickening is not clear further than that it is out of it that the epibranchial placode forms. The epibranchial placodes and their relation to the visceral ganglion have been fully discussed in a previous paper (Landacre '12).

6. The post-auditory lateral line primordia appear in three divisions, the most anterior of which and the first to appear lies in a longitudinal axis on a level with the thickening of the first true gill. In the dorso-ventral axis it lies dorsal to that thickening. It appears almost simultaneously with the appearance of the operculum and its anterior end lies over the anterior attached border of the fold and it extends from this point back over the first gill thickening. This lateral line primordium appears at least twelve hours after the complete detachment of the auditory vesicle from the epidermis.

While in a flat reconstruction, such as figure 4, its relation to the vesicle seems to be close, it is really well detached from the ear on account of the distance between the epidermis and the vesicle. In view of the absence of a postauditory placode, there is no reason, in the opinion of the writers, for relating this primordium genetically with that of the auditory vesicle. As mentioned in the body of the paper, the posterior end of this primordium comes quite close to the lateral line ganglion of the IX nerve, suggesting the origin of that ganglion from the lateral line primordium, although our stages are not taken sufficiently close together to settle this question. The lateral line organs which differentiate later in this primordium are supplied by the ramus supratemporalis IX.

The two remaining primordia appear later than the one just described and are more posterior in position. Both appear for the first time in the same series, the more anterior being located over the second gill thickening and the more posterior extending from a point over the third gill thickening back over the fourth and on over the position of the unformed fifth gill thickening, ending some distance behind this point with its posterior end at a somewhat lower level than its anterior end.

The more anterior of these two thickenings, or the second, has the same general relations to the second gill thickening that the first lateral line primordium has to the first gill thickening. It is more dorsal in position and has, soon after its appearance, definite anterior and posterior limits and also soon shows the characteristic radial arrangement of cells which is so usual in lateral line primordia.

Its relation to the lateralis X ganglion is quite similar to the relation of the first lateral line primordium to the lateralis IX ganglion, that is, it is closely in contact with the ganglion at its posterior end and suggests the origin of this ganglion from the primordium of the lateral line. The anterior end of the lateralis X ganglion appears further to be distinct from the longer posterior portion, indicating the presence of two lateral line ganglia on X. The lateral line organs differentiated in this primordium receive their fibers from the ramus supratemporalis X nerve.

Since both the second and third lateral line primordia appear in the same series, a question might be raised as to whether the second might not have been found connected with the third in a closer series. In view of the number of separate lateral line primordia in the preauditory region and in view of the fact that the first postauditory primordium is certainly not connected with the second and third, the question is not of vital significance. The facts mentioned above, in addition to the amount of undifferentiated ectoderm lying between the second and third primordia, are evidence against their continuity in earlier stages.

The third postauditory lateral line primordium at the time of its appearance is the largest of the three and grows backward rapidly along the side of the body, and on it differentiate the or-

gans of the main body line. These organs are all supplied by the ramus lateralis X. Whether the accessory organs found later lying above the main lateral line arise by splitting off from that line could not be determined. These are supplied, however, from the same ramus. The relations of this third primordium to the lateralis X ganglion are quite similar to those of the two more anterior to their respective ganglia. The posterior end of the ganglion comes so close to the lateral line primordium as to suggest its derivation from that primordium, but here again our series are not close enough together to settle the question.

The third primordium, as in the case of the other two, is more dorsal in position than the gill thickenings and quite distinct from them, although at the time of its appearance its posterior end drops to the level of the gill thickenings. This is, however, at a point back of the region in which the last gill thickening appears.

The definite mode of appearance of the postauditory lateral line primordia, the absence of a postauditory placode, and especially the presence of a number of gill thickenings which might easily be mistaken for either of the two preceding thickenings, but whose history has been traced, furnish positive evidence against the theory that the lateral line primordia are derived genetically from the auditory vesicle.

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ABBREVIATIONS

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|--|--|
| <i>Ang.Th.</i> , thickenings at the angle where the vertical lateral body wall becomes horizontal | <i>Ec.Th.</i> , 1, 2, 3, ectodermal thickenings lying in the region of and extending behind the first, second and third true gills |
| <i>Aud. V.</i> , auditory vesicle | <i>E.P.VII</i> , epibranchial placode of VII |
| <i>Con. 1, 2 and 3</i> , contact of the endodermic gill pocket of the first, second and third true gills with the ectoderm | <i>E.P.IX</i> , epibranchial placode of IX |
| <i>D.L.VII</i> , dorso-lateral VII ganglion | <i>Epi.</i> , epiphysis |
| <i>Ec.Th.</i> , ectodermal thickening in the region of the gill contact | <i>Gass.</i> , Gasserian ganglion |
| | <i>G.VII.</i> , general visceral portion of VII |
| | <i>Hy.Con.</i> , hyoid contact of ectoderm and endoderm |

<i>Hyp.</i> , hypophysis	<i>Po.LL.</i> 1, 2, 3, the first, second and third postauditory lateral line primordia
<i>Lat.IX</i> , lateralis ganglion of IX nerve	<i>Pr.A.P.</i> , preauditory placode
<i>Lat.X</i> , lateralis ganglion of X nerve	<i>Pr.L.L.</i> , primordium of sensory lines anterior to auditory vesicle
<i>Mes.</i> , mesencephalon	<i>Prc.</i> , prosencephalon
<i>Met.</i> , metencephalon	<i>Sub.L.</i> , sub-orbital line
<i>Md.L.</i> , hyo-mandibular line	<i>Sup.L.</i> , supra-orbital line
<i>Olf.C.</i> , olfactory capsule	<i>Vis.IX</i> , visceral ganglion of the IX nerve
<i>Opt.V.</i> , optic vesicle	<i>Vis.X</i> , visceral ganglion of the X nerve
<i>P.E.P.</i> , posterior extension of epibranchial placode of VII	
<i>Ph.</i> , pharynx	
<i>Po.A.P.</i> , postauditory placode	
<i>Po.L.L.</i> , primordium of lateral lines posterior to auditory vesicle	

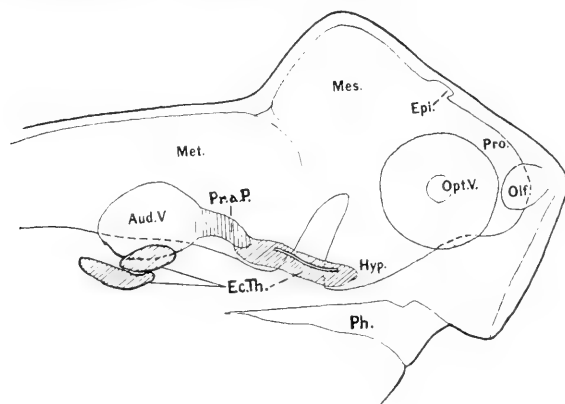
Figures 1 to 6 are flat reconstructions of *Lepidosteus osseus* made at a magnification of 75 diameters. In all six figures the brain and sense organs are shown in outline only. The ectodermal gill thickenings are shown in diagonal lines; the actual area of contact between ectoderm and endoderm in the hyoid region by an open line; the epibranchial placode by stipple; the primordia of the lateral lines by cross-hatching and the preauditory placode by vertical lines.

PLATE 1

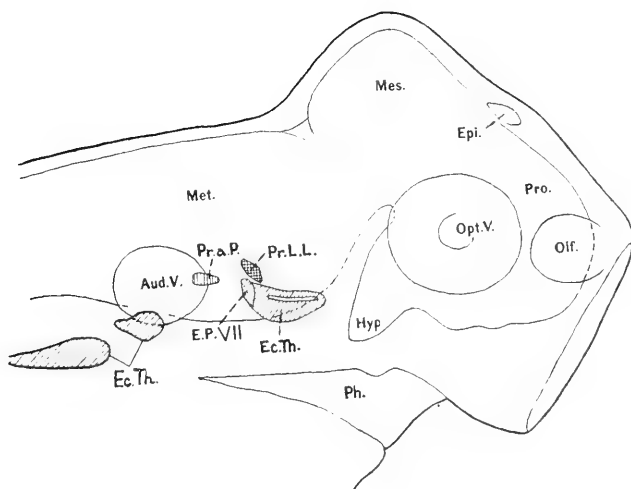
EXPLANATION OF FIGURES

1 Reconstruction from Stage XVI, age 88 hours. The preauditory placode is in this stage a cephalad continuation of the auditory vesicle. The ectodermal thickening in the region of the hyoid contact is shown, continuous posteriorly with the preauditory placode. In the postauditory region two thickenings are present. The more dorsal is associated with the first gill and the more ventral with the second gill.

2 Stage XVII; age 94 hours; shading and abbreviations as in figure 1. The auditory vesicle is completely detached from the ectoderm and the preauditory placode. The placode is quite small and overlaps the auditory vesicle and extends three sections (30μ) only in front of it. The epibranchial placode is present and just dorsal to it lies the primordium of the supra- and infra-orbital lines. In the postauditory region, the first gill thickening is completely detached from the second which extends considerably farther caudad:



1



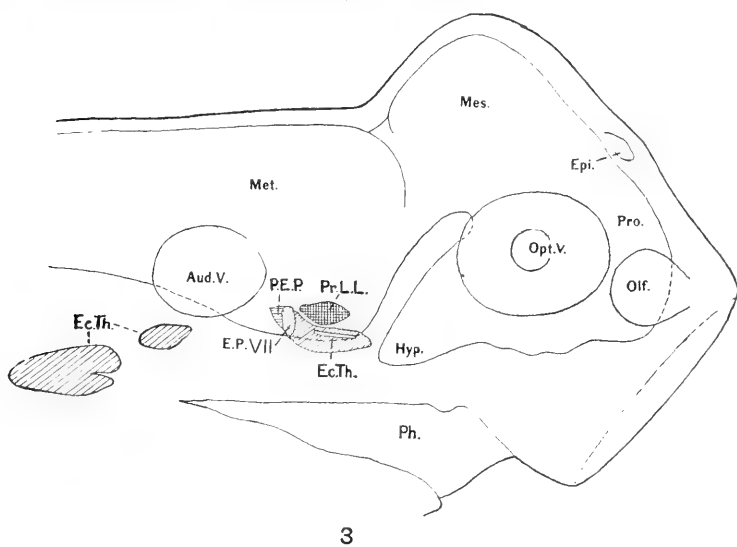
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PLATE 2

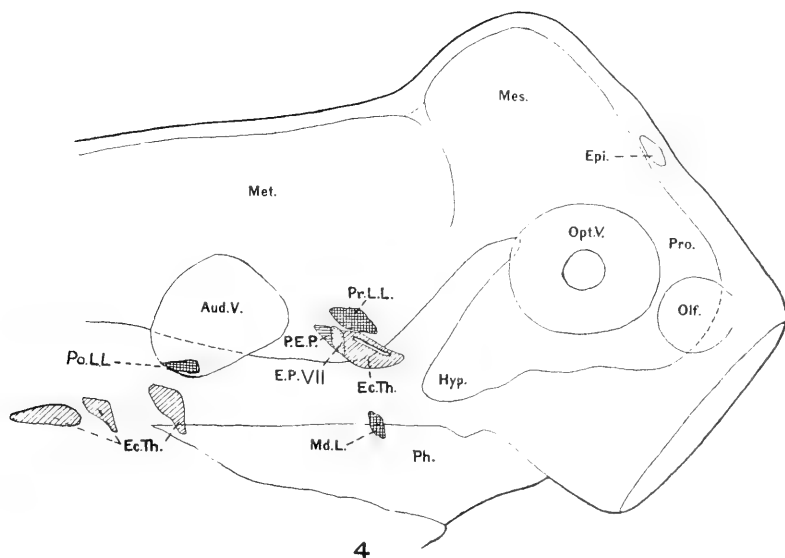
EXPLANATION OF FIGURES

3 Reconstruction of Stage XVIII, age 100 hours; length 7 mm. shading and abbreviations as in figure 1. In the preauditory region a thickening has appeared behind the epibranchial placode (horizontal shading). Since the placode appeared earlier than the thickening, it is called in the description of this region the posterior extension of the epibranchial placode, but it seems to be homologous with the extensions behind the gill thickenings of the true gills with the exception that they appear before the epibranchial placodes. The preauditory placode is absent and the lateral line primordium is larger than in figure 2. In the postauditory region the second ectodermal gill thickening is split at its anterior end into two portions of which the more dorsal represents the posterior extension of the second gill and the more ventral the area of contact of the third gill. The posterior portion of this second thickening is the angular thickening.

4 Reconstruction of Stage XIX; age 106 hours; length 7.3 mm.; shading and abbreviations as in figure 1. This stage shows the first trace of the primordium of the mandibular lateral line, also the first trace of the postauditory lateral line. There are present three gill thickenings posterior to the ear associated with the first, second and third gills. The posterior extension of the third thickening represents the angular thickening.



3



4

PLATE 3

EXPLANATION OF FIGURES

5 Reconstruction of Stage XXI; age 120½ hours; length 8.5 mm.; shading and abbreviations as in figure 1. In this stage the supra-orbital, infra-orbital and mandibular lines have grown forward almost to the optic vesicle. The epibranchial placode has reached nearly its maximum size and projects into the mesoderm, but its point of attachment is not shown in this plot. In the postauditory region there are three lateral line primordia, one over the first gill, one over the second gill thickening and a third beginning over the third gill thickening and extending back from this point. The last is the primordium of the main lateral line. There are four gill thickenings, the first three extending behind the first three gills and the fourth lying in the region of contact of the fourth endodermic gill pocket.

6 Reconstruction of the preauditory region of Stage XXVI; age 154 hours; length 10 mm. The extent of the preauditory lines at this age is shown, as well as the persistence of the hyoid ectodermal thickening.

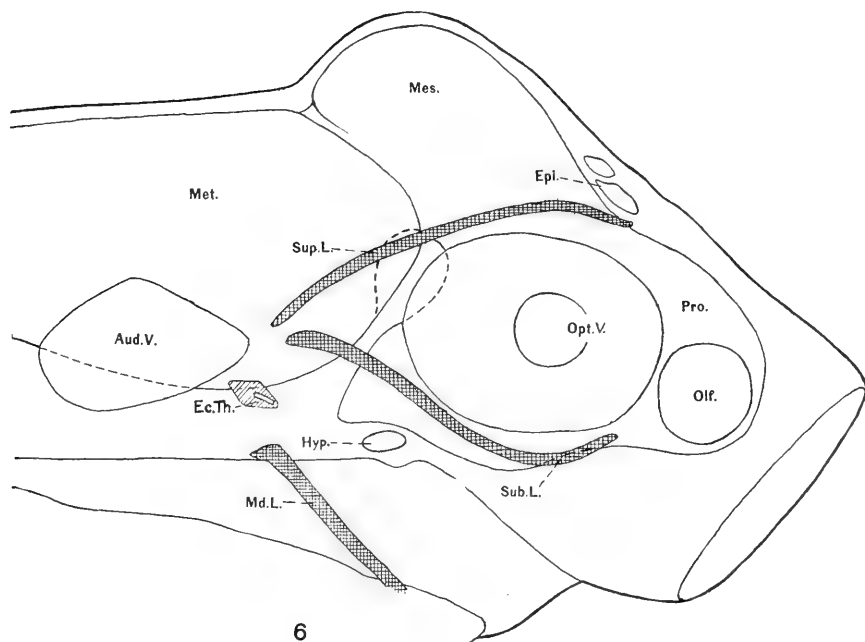
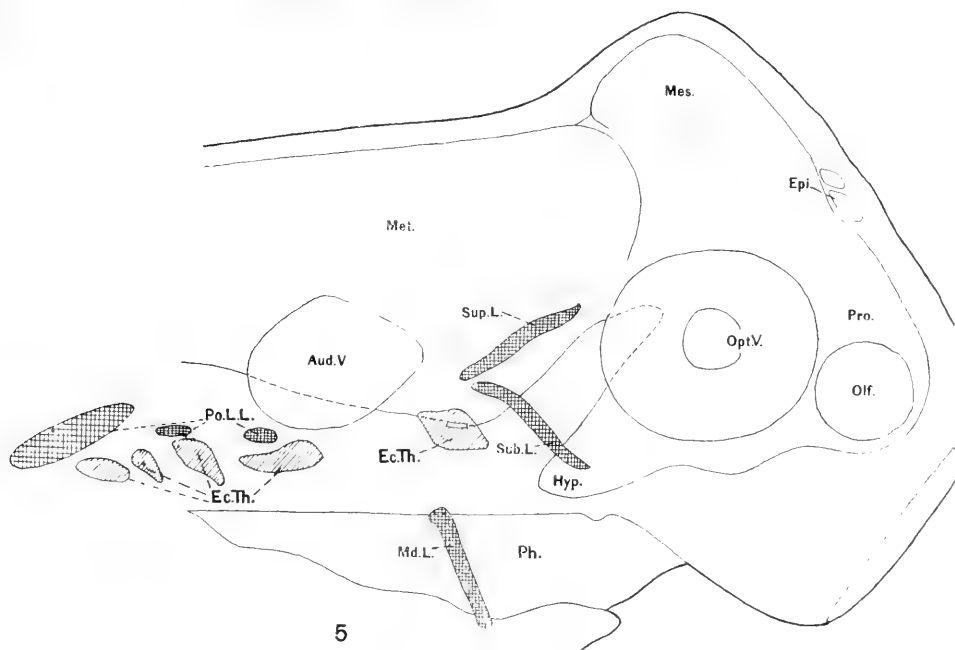


PLATE 4

EXPLANATION OF FIGURES

7 Shows the detail of the first gill thickening in a 76-hour embryo. The section lies anterior to that of figure 27 just behind the area of contact of the first endodermic gill pocket. $\times 300$.

8 From a 100-hour embryo, shows the primordium of the supra-orbital lines, and its relation to the posterior extension of the epibranchial placode. $\times 300$.

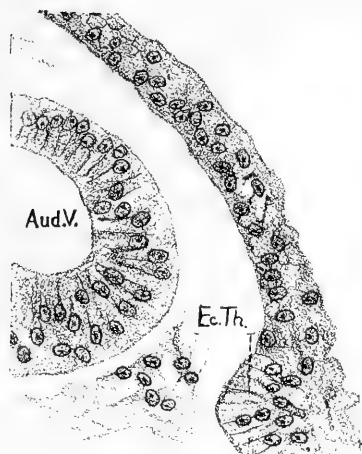
9 to 12 Drawn from an embryo of 137 hours, in which the development was below normal, so that they illustrate conditions prior to the $120\frac{1}{2}$ -hour stage. $\times 300$.

9 Drawn through the primordium of the lines, and shows the characteristic cell arrangement.

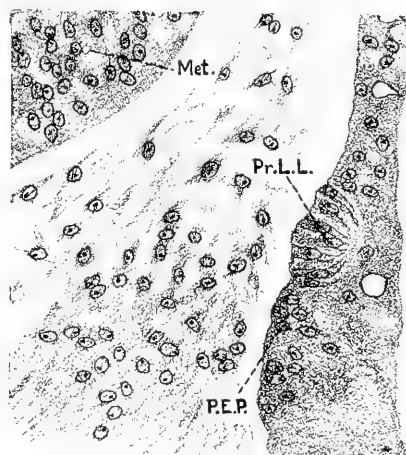
10 Drawn from the next section anterior to figure 9, and exhibits the first appearance of the bifurcation of the anterior end of the primordium.

11 Taken two sections anterior to figure 10, and is the first section to show a complete bifurcation.

12 Drawn from the next section anterior to figure 11. This shows the two lines (supra-orbital and sub-orbital) are diverging at this point, as will be seen by comparing figures 11 and 12.



7



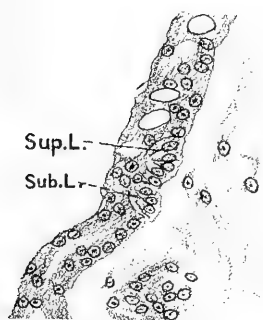
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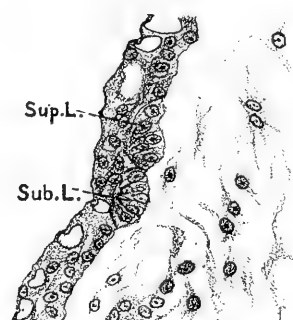
9



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PLATE 5

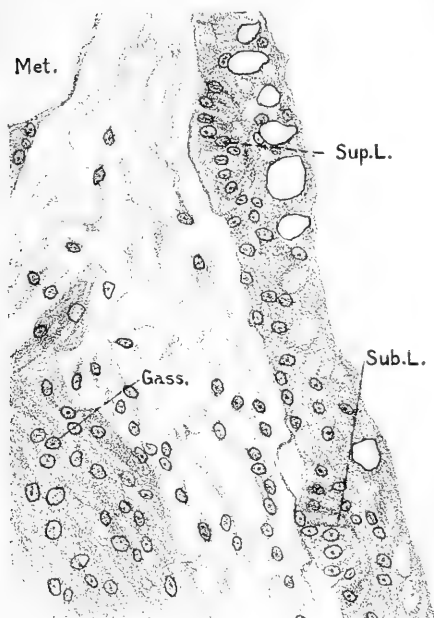
EXPLANATION OF FIGURES

13 From 120½-hour embryo, shows the supra-orbital and sub-orbital lines at the level of the Gasserian ganglion. $\times 300$.

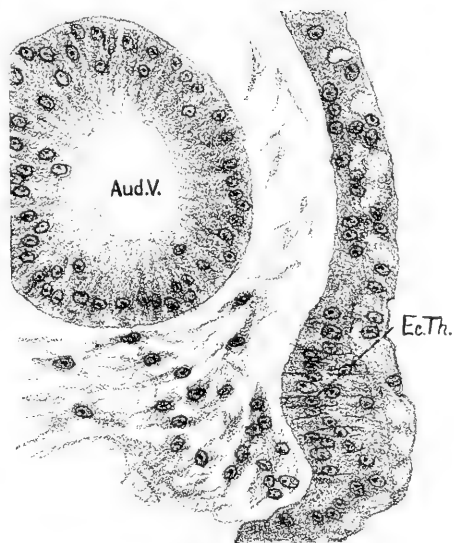
14 Shows the detail of the posterior extension of the first gill thickening in an embryo of 94 hours. $\times 300$.

15 From 120½-hour embryo, is taken four sections posterior to the section shown in figure 13. The two lines are converging and the radial arrangement is not so pronounced as in sections through more anterior points. $\times 300$.

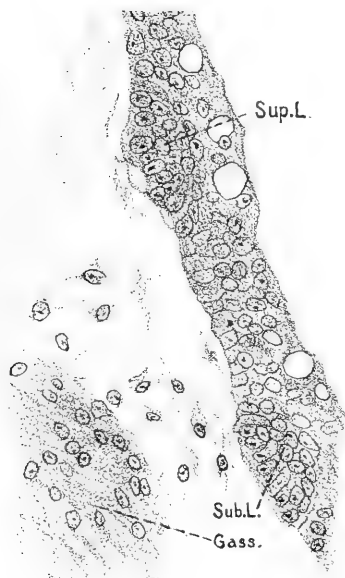
16 From a 120½-hour embryo; shows the general relations of structures in the region of the hyoid contact. This section shows the epibranchial placode, lying just lateral to the hyoid contact and the primordium of the supra-orbital line, situated dorsal to the epibranchial placode. The histological characters which serve to distinguish the ectodermal thickening and the epibranchial placode from the sensory line primordium are shown in this section. This section is five sections posterior to that shown in figure 15. $\times 300$.



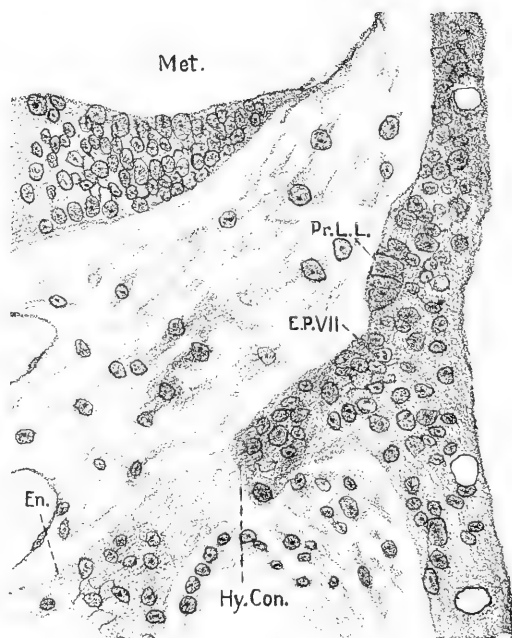
13



14



15



16

PLATE 6

EXPLANATION OF FIGURES

17 From a 76-hour embryo; shows the detail of the preauditory placode, and its resemblance to the auditory vesicle in early stages. This figure is taken five sections in front of the vesicle. $\times 300$.

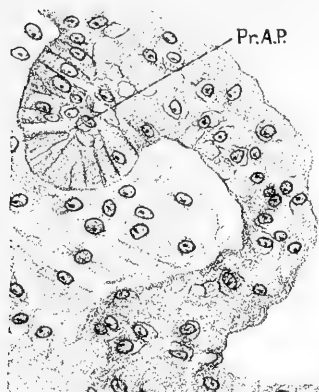
18 Shows the auditory vesicle and the first gill thickening in a 100-hour embryo. The overlapping of the thickening by the vesicle is shown in this section. $\times 300$.

19 Shows the detail of the preauditory placode in an 82-hour embryo. The general reduction in the size of the placode and the gradual loss of radial cell arrangement may be seen by a comparison of figures 1 and 22. $\times 300$.

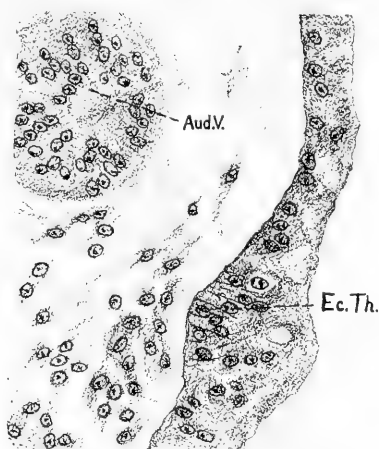
20 From a 120½-hour embryo, shows the mandibular line. The radial arrangement of the cells is not so apparent as in the other lines. $\times 300$.

21 Shows the appearance of the primordium of the supra-orbital and infra-orbital lines combined in a 106-hour embryo. This section also shows that the sensory line primordium lies at a more dorsal level than the posterior extension of the epibranchial placode. $\times 300$.

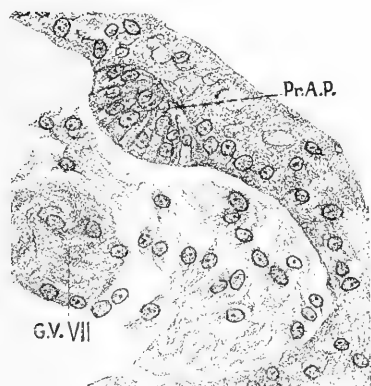
22 From an 88-hour embryo, shows the appearance of the preauditory placode. This section passes through a point two sections behind the anterior limit of the placode. Compare with figures 17 to 19 which are taken at a more anterior level from earlier embryos. $\times 300$.



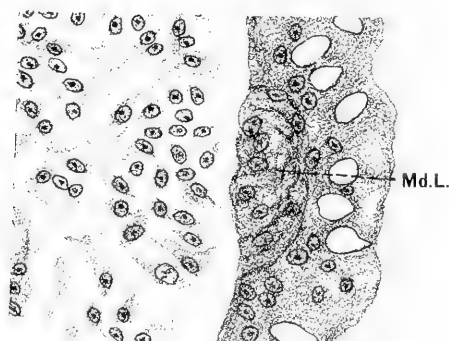
17



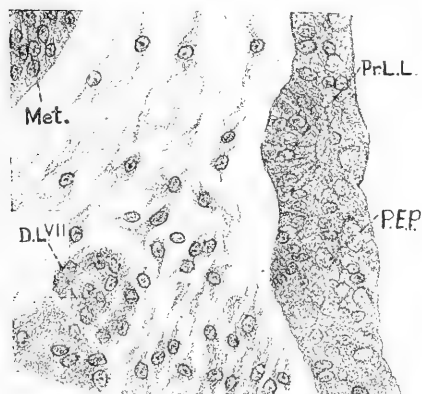
18



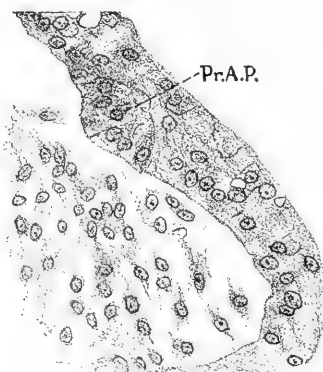
19



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PLATE 7

EXPLANATION OF FIGURES

23 Stage XIII, age 72 hours. A camera outline taken through the middle of the contact of the endodermic pharyngeal pocket of the first true gill with the ectoderm. At this point the ectoderm is not thickened. $\times 75$.

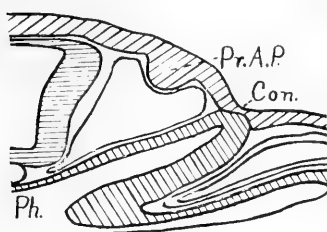
24 Stage XIII, age 72 hours. A camera outline at the posterior limit of the area of contact of the endodermic pharyngeal pocket of the first true gill with the ectoderm. The ectoderm is thickened at the point of contact. $\times 75$.

25 Stage XIII, age 72 hours. A camera outline taken behind the area of contact of pharyngeal endodermic pocket of the first true gill and ectoderm. The section lies near the anterior end of the cavity of auditory vesicle. The epidermis is only slightly thickened in this section, but the thickening is continuous with that shown at the same level in figure 24. $\times 75$.

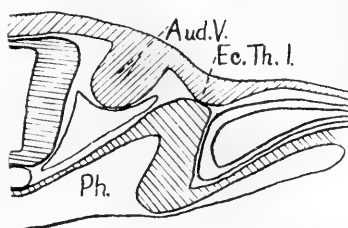
26 Stage XIII, age 72 hours. A camera tracing taken one section anterior to the posterior end of the auditory vesicle. The gill thickening extending through the two preceding series cannot be located at the level of this section. The vesicle lies much closer to the neural canal than to the ectoderm. $\times 75$.

27 Stage XIV, age 76 hours. A camera outline taken posterior to the area of contact of the pharyngeal pocket to the first true gill with the ectoderm. The gill thickening shown in the four preceding figures reaches further ventrally and is continuous with the angular thickening, but no indication of a separation can be seen in this stage. $\times 75$.

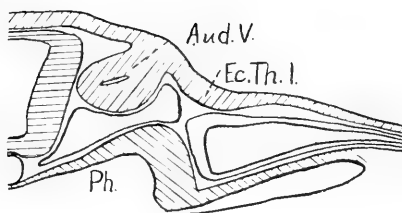
28 Stage XV, age 82 hours. A camera outline taken just behind the area of contact of the pharyngeal pocket of the first true gill. The separation of the common thickening shown in figure 27 has progressed in this stage. The more ventral or angular thickening represents the area of contact of the second true gill pocket. $\times 75$.



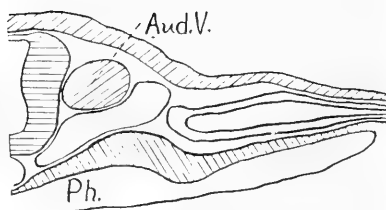
23



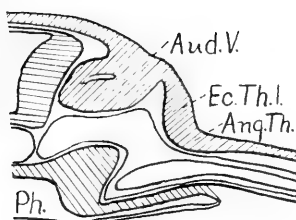
24



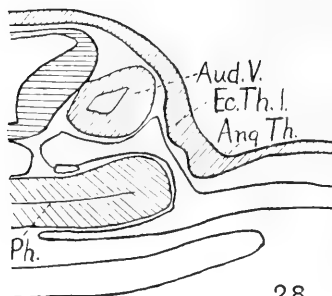
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PLATE 8

EXPLANATION OF FIGURES

29 Stage XVI, age 88 hours. A camera outline taken through the contact of the second true gill with the ectoderm. The posterior extension of the first true gill thickening, *Ec.Th. 1*, is pronounced in this series. The second endodermic gill pocket touches the more ventral thickening which in figure 28 is labelled *Ang.Th.* This section lies, in the longitudinal axis, near the middle of the two thickenings of figure 1, at which point they overlap.

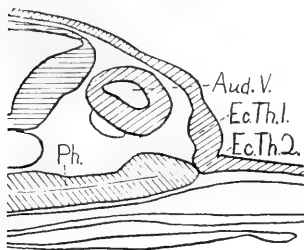
30 Stage XVII, age 94 hours. A camera outline taken through the area of contact of the second true gill pocket, and the anterior end of the angular thickening. The section is taken through the anterior end of the second postauditory ectoderm thickening of figure 2. The region marked *Ang.Th.* in figure 30 is actually no thicker than the intermediate region lying between it and the area of contact, *Ec.Th. 2*, but it is in the exact position of the angular thickening (*Ang.Th.*) of figure 31. $\times 75$.

31 Stage XVIII, age 94 hours. A camera outline just behind the area of contact of the second true gill pocket. The third gill pocket is not yet in contact with the ectoderm and the more ventral thickening is consequently labelled angular thickening (*Ang. Th.*), but it represents the area of contact of the third true gill pocket. $\times 75$.

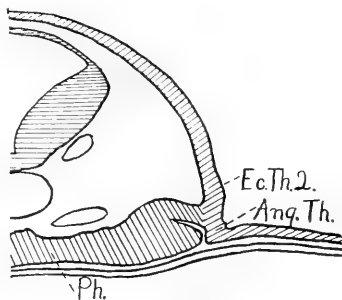
32 Stage XXI, age 120½ hours. A camera outline taken near the posterior end of the auditory vesicle, and behind the first gill slit in the posterior extension of the thickening of this slit, *Ec.Th.1*. The primordium of the lateral line, *Po.L.L.* is close to the lateralis IX ganglion, but not so close as at the posterior end of the primordium. For note on figures 32 and 5, see next paragraph. $\times 75$.

33 Stage XXI, age 120½ hours. A camera outline taken through the extreme anterior end of the lateralis X ganglion and the extreme posterior end of the thickening extending behind the second gill. There seems to be discrepancy between figures 32 and 33 and figure 5, in that figures 32 and 33 show a gill contact overlapping the preceding lateral line primordium, while in figure 5, for instance, the first lateral line primordium does not overlap the second gill contact. This apparent discrepancy is due to the fact that in figure 5, as in all reconstructions (figs. 1-6) gill contacts are not shown on the plot unless there is a noticeable thickening of the ectoderm in the region of contact. The contacts (*con.2* and *con.3*) on figures 32 and 33 are contacts simply with no thickening of the ectoderm. $\times 75$.

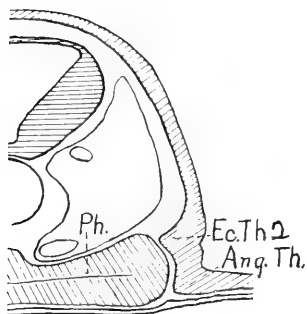
34 Stage XXI, age 120½ hours. Camera outline taken at posterior end of lateralis X ganglion and behind the fourth gill contact of figure 5. This section lies near the posterior end of the thickening extending back from the fourth gill contact and, since the fifth gill contact is not yet found, is labelled angular thickening. $\times 75$.



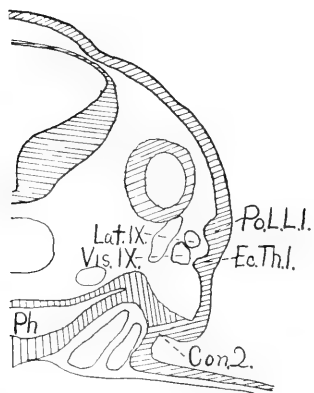
29



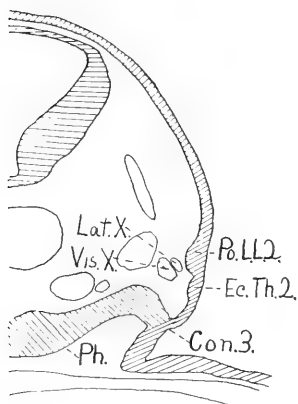
30



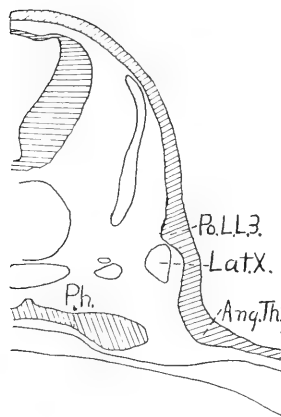
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NOTES ON THE ANATOMY OF A CYCLOSTOME BRAIN: ICHTHYOMYZON CONCOLOR

C. JUDSON HERRICK AND JEANNETTE B. OBENCHAIN

Anatomical Laboratory of the University of Chicago

TWELVE FIGURES

In the literature relating to the brain of *Petromyzon* and its allies there are accounts of the external form of the brain in several of these species; but the only full descriptions of the sculpturing of the ventricular surfaces in correlation with the underlying internal structures are found in the excellent recent paper by Johnston ('12). A brief reference to some of these structures in the lake lamprey, *Ichthyomyzon concolor* (Kirtland), was published by Herrick ('10, p. 470) on the basis of a single series of transverse sections of a specimen 120 mm. long from Lake Erie. Johnston, finding some features of this description out of harmony with his observations upon other species of petromyzonts, reexamined this series of sections and constructed a plate model of the right half of the forebrain. A figure of the mediâl view of this model magnified 66 diameters was published in his paper cited above.

In view of the discrepancies between these two descriptions of the same material, we have undertaken the study of a second specimen of this species. The first specimen was obtained, sectioned and loaned to us by Dr. Charles Brookover. The second specimen, also very kindly put at our disposal by Dr. Brookover, came from the same lot as the first and is somewhat larger, measuring 140 mm in length. Both are young adults. The entire head was cut into transverse sections 15 μ thick and stained with iron hematoxylin and acid fuchsin. Drawings of the brain were made from these sections with the aid of the Edinger projection

apparatus and transferred to wax plates, from which a model of both sides of the brain was constructed magnified 75 diameters.

This model includes the entire brain back to a point between the superficial origins of the Vth and VIth cranial nerves, save for the omission of the choroid plexuses of the mid-brain and medulla oblongata. In the telencephalon and diencephalon thin wax plates were used which were almost exactly seventy-five times the thickness of the sections, so that almost every section is represented in the model, which preserves in this region the ventricular sculpturing with all possible accuracy. As the plates were stacked, measurements were made with callipers after the addition of each group of five plates and thus the cephalo-caudal length of the model was controlled by comparison with the computed length required by the magnification chosen. This control required the omission of two plates, one in front of the lamina terminalis and one in front of the posterior commissure. With these two exceptions the model contains all of the sections of the series from the anterior end of the brain to a point behind the posterior commissure. Behind this level somewhat thicker plates were used. This, however, involves no loss in accuracy, since the superficial structures of the mid-brain and oblongata are larger and more easily defined than are those of the thalamus. After carefully stacking the plates, they were cemented sufficiently to hold them together. The right and left halves were then cut apart in the medial plane with a silk thread and the halves strengthened with wire stays sunk into the cut surfaces of the wax only, so that the ventricular surface, which presented a very good appearance, might be studied unaltered in any detail. The ventricular surfaces were left untouched until after the completion of the study, which included a careful microscopic examination of all of the sections to determine what deep structures are related to each superficial eminence and groove.¹

A dorsal view of the model is seen in figure 1.

¹ The labor of preparing and drawing the sections, transferring the drawings to the wax plates and cutting the plates was performed entirely by Miss Obenchain, who at that time had not studied the material nor familiarized herself with the morphological problems involved. The plates, as stated above, were stacked be . .

EXTERNAL SURFACE

Figure 2 shows the external form of the right side of the model. In front of the superficial origin of the Vth nerve is a sharp constriction, the isthmus, which separates the medulla oblongata and cerebellum behind from the midbrain in front. It is occupied dorsally by the decussation of the IVth nerve.

The roof of the mid-brain is membranous and plexiform save for a post-tectal commissure immediately in front of the cerebellum and the posterior commissure at the diencephalic boundary. The attachment of this choroid plexus to the massive wall is termed the taenia mesencephali (figs. 1 and 12, *t.m.*). The lateral wall of the mid-brain is marked by two very prominent eminences, a tectal eminence (*tect.*) dorsally and a peduncular eminence ventrally (*ped.*). Between these is a less prominent tegmental area (*tegm.*). On the ventricular surface the sulcus limitans of His marks the boundary between the tegmental and peduncular regions (cf. figs. 3 and 4). The superficial origin of the III nerve marks the anterior border of the peduncular eminence. The tectal eminence is formed by a lateral evagination involving the entire thickness of the brain wall, thus producing a dilation of the ventricle, the optocoele (fig. 4). The tegmental eminence, on the other hand, is a solid thickening of the wall which extends somewhat farther caudad than the tectum. The ventricle is pushed downward to form a distinct ventral recess in this region, but is not laterally dilated (fig. 4).

The position of the posterior commissure is indicated by a distinct eminence in front of the tectum on both the lateral and the ventricular surfaces (figs. 2, 3, *em. pc.*), which extends

fore being cut into right and left halves entirely with reference to the external features of the brain, so that neither of the authors was able to see the ventricular surfaces until after the stacking was finished and the completed model cut in half. The observance of these precautions insures an objective result, so far as the ventricular surfaces are concerned. The subsequent microscopic study of the sections and their interpretation was done jointly by the two authors, though the senior author assumes entire responsibility for the terminology adopted and for the discussion which forms the final section of the report.

almost as far ventrally as the locus of the sulcus limitans. The tuberculum posterius of the mid-brain extends considerably farther rostrad than the level of the posterior commissure and is marked by an external eminence immediately dorsally of the mammillary body (fig. 2, *t.p.*). The rostral boundary of this eminence and of the mammillary body is a well defined sulcus which separates these structures from the large eminence formed by the optic chiasma and preoptic nucleus above and the hypothalamus below. Immediately above the attachment of the optic nerve is a small, relatively depressed region within which is the nucleus olfactorius medialis (*nuc. ol.m.*).

At the ventral end of the chiasma ridge and immediately above it is a narrow deep membranous evagination of the brain wall, the preoptic recess (figs. 2, 3, *r.po.*). This projects slightly forward to form the most rostral part of the brain except the olfactory bulb. There is a wider and shallower postoptic recess below the chiasma ridge (fig. 3, *r.o.*) which, however, is not externally evident in a lateral view of the brain. The postoptic recess is bounded behind by a groove on the ventral surface of the brain, which is not externally visible because it is covered by the pars glandularis of the hypophysis. It is a conspicuous object in the medial section of the brain (fig. 3, *com.pri.*), where its floor is seen to be occupied by the commissura preinfundibularis (p. 653). In the floor of the ventral groove which marks the rostral boundary of the mammillary body there is also a commissure, the commissura postinfundibularis (fig. 3, *com.pi.*).

The corpus mammillare is a deep evagination of the massive brain wall (fig. 2, *c.mam.*) directed ventralward and backward and containing the recess mammillaris (figs. 3, 4, *r.mam.*). This structure is unpaired, but asymmetrical, the right side being much larger than the left.

Between the postoptic recess and the corpus mammillare the ventral part of the third ventricle is widely dilated laterally to form a thin-walled infundibular sac. This sac forms the most ventral part of the brain, the rostral portion of its walls being concealed in surface views of the brain by the glandular part of the hypophysis. This portion is the pars nervosa of the hypophy-

sis. The posterior third of this sac is entirely free from contact with the glandular part of the hypophysis and probably is comparable with the free saccus vasculosus of some teleostean fishes and urodele amphibians, as has been pointed out by Schilling ('07).

The glandular part of the hypophysis spreads over the anterior two-thirds of the infundibular sac and forward to the preoptic recess, the rostral end of the mass being thicker than the caudal end. It is closely applied to the nervous wall except for a short distance in the groove under the preinfundibular commissure. Its swollen anterior end projects forward beyond the preoptic recess, from which, however, it is separated by a deep sulcus.

The most conspicuous difference between the right and left halves of the brain is due to the asymmetry of the habenulae and the parts immediately related thereto. The right habenula is several times larger than the left; it does not, however, overreach the mid-plane, as it does in the 120 mm. specimen figured by Johnston.

The right habenula covers dorsally the entire thalamus, extending from the pineal recess in front of the posterior commissure forward to the primordium hippocampi, whose posterior end is, in fact, overlapped by the free anterior tip of the habenula within the dorsal sac (fig. 4). The habenula is bounded below on the lateral aspect by a sharp sulcus subhabenularis. This sulcus appears on the medial surface also.

The anterior third of the habenula is intra-ventricular, being enveloped laterally by the membranous dorsal sac, whose line of attachment (taenia thalami) is seen in figure 1. There is a small sharply defined eminence under the free anterior tip of the habenula, which is visible from both the lateral and the ventricular surfaces. It lies below the taenia thalami and is continued backward into the substance of the habenula and forward into the fimbria. It is full of small cells similar to those of the habenula and of fibers of the fimbria system from the primordium hippocampi to the habenula. This may be termed the eminentia fimbriae (figs. 2, 3, *em.f.*) and appears to be merely a portion of the habenula which has been differentiated in connection with the

dorsal portion of the stria medullaris complex which passes through the primordium hippocampi (tractus cortico-habenularis medialis, etc., see beyond). This is the structure which is marked *em.th.* in figure 9 of Johnston's paper ('12, p. 381), and which is not the same thing as the structure similarly marked in some of his other figures (p. 660).

Immediately ventrally of the subhabenular sulcus is another eminence of the lateral surface, the lobus subhabenularis thalami, which is also visible on the ventricular surface (figs. 1, 2, 3, *l.sh.*). This eminence is separated by a very shallow external groove from a similar elevation farther forward, the primordium hippocampi, most of which is concealed in the stem-hemisphere fissure (fig. 2). All these features related to the habenula are much more prominent on the right than on the left.

The dorsal sac is much larger and more widely dilated in this specimen than in Johnston's model of the 120 mm. specimen. Its highest point is at the junction of the pineal stalk and vesicle above the anterior end of the right habenula.

The pineal vesicle is large and approximately circular in outline as seen from the dorsal surface (fig. 1, *ep.*). A small parapineal organ is apparently concealed beneath the pineal vesicle; but, as the tissues of this region were poorly preserved, no attempt was made to model it. A slender solid pineal stalk extends backward from the pineal vesicle to a point above the pineal recess. The size of this stalk is somewhat exaggerated in the model.

The wall of the dorsal sac becomes continuous forward with the lamina supraneuroporica within which is the dorsal commissure (fig. 4). Above this commissure is a transverse fold or wrinkle of the membranous roof (figs. 2, 3, *v.tr.*) which probably represents the velum transversum, forming the boundary between the dorsal sac and the lamina supraneuroporica. This fold extends only a short distance lateralward and then fades out in the wall of the dorsal sac. Its ventral (rostral) limb laterally joins the massive primordium hippocampi along the taenia fornicis (fig. 5).

Each cerebral hemisphere is divided by a deep obliquely transverse sulcus into two unequal parts; the larger anterior part is the olfactory bulb and the posterior part is the secondary olfactory area, or lobus olfactorius (figs. 1, 2, *l.ol.*). These parts are separated from the telencephalon medium by a sharp stem-hemisphere fissure except along the ventral border. Here the transverse sulcus also disappears, this region being occupied by a ventral eminence containing dorsally the primordium of the corpus striatum and ventrally the nucleus olfactorius medialis.

The forebrain of *Ichthyomyzon* is more compressed in the cephalo-caudal direction than are the brains of most other petromyzonts which have been described, though the model of the 140 mm. specimen is somewhat less so than is Johnston's model of the 120 mm. specimen (cf. our figure 4 with his figure 6) the ratio of length to height in his model being 48 : 100 and in our model 58 : 100, leaving out of consideration the dorsal sac in both cases.

MEDIAL SURFACE

Figure 4 illustrates the ventricular surface and the cut surfaces of the right half of the divided model, and figure 3 is a key diagram to indicate the relations of the ventricular eminences and sulci and some of the underlying structures.

The cerebral ventricle in front of the isthmus is high in dorso-ventral diameter, but very narrow in transverse diameter except in a few places where lateral evaginations of the whole wall cause shallow or deep pockets on the ventricular surface. These evaginations are as follows: (1) the tectum mesencephali, marked on the ventricular surface by the optocoele; (2) the metathalamic recess; (3) the pineal recess; (4) the dorsal sac; (5) the cerebral hemisphere; (6) the preoptic recess; (7) the postoptic recess; (8) the lower part of the infundibulum (saccus vasculosus of Johnston); (9) the mammillary recess. The remaining parts of the ventricular surface show a definite sculpturing in low relief, whose sulci do not involve the entire thickness of the brain wall.

In the anterior part of the medulla oblongata there are two longitudinal sulci, a shallow more medial groove separating the somatic motor column from the lateral or visceral motor column and a very deep lateral sulcus separating the lateral motor column containing the motor V nucleus from the somatic sensory column containing the area acustica and the sensory V nucleus. The lateral sulcus is the sulcus limitans of His, whose further relations will next be considered.

Sulcus limitans of His. The position of this sulcus can be readily followed from the medulla oblongata to the level of the tuberculum posterius at the rostral border of the mid-brain. Under the cerebellum the strong eminence formed by the motor V nucleus flattens out and the sulcus limitans becomes a wide shallow groove which bends upward above the fifth pair of giant cells of Müller (fig. 3). In the caudal end of the mid-brain the sulcus disappears for a short distance, but its morphological position can be clearly determined from internal evidence. In this part of its course it descends to a point above the fourth pair of Müller cells and from here forward again is externally visible as a shallow but sharply defined groove. Under the posterior commissure it rises abruptly to clear the third pair of Müller cells, in front of which it drops again. In front of the first pair of Müller cells it ascends gently to a point above the anterior end of the tuberculum posterius. Here it disappears behind the lobus ventralis thalami.

There is a deep sharply defined depression above the posterior end of the chiasma ridge which is termed sulcus medius thalami. The posterior end of this depression lies in line with the stretch of the sulcus limitans last described, though it is separated from it by the lobus ventralis thalami, and internal evidence suggests that it is a forward continuation of the sulcus limitans of His. Under the caudal end of the sulcus medius there is a depressed area (more obvious on the left side than on the right) extending downward and forward into the recessus preopticus which probably represents the anterior end of the sulcus limitans.

Reviewing the sulcus limitans as a whole, we find it very deep in the anterior end of the medulla oblongata, shallow and wide

under the cerebellum and quite absent superficially in the posterior part of the mesencephalon. It reappears as a very slight groove in the anterior part of the mid-brain and posterior part of the thalamus. It is interrupted in the mid-thalamic region by the lobus ventralis thalami, and may be represented farther forward in the sulcus medius and a wide shallow depression extending from the later into the preoptic recess.

This sulcus separates a ventro-lateral motor lamina from a dorso-lateral sensory lamina of the neural tube, and throughout the medulla oblongata the type of cellular differentiation of these two laminae is very characteristic. In the mid-brain these differences, though less conspicuous, are still evident. The ventral lamina is characterized by the giant cells of Müller, of which five pairs fall within the limits of our model (fig. 3). Even when these cells are left out of account, the motor lamina exhibits larger and more irregularly arranged neurones than the sensory. Throughout the mid-brain, even where the limiting sulcus is not evident on the ventricular surface (fig. 12), this difference in the character of the neurones, reinforced by a thickening of the ependyma in the site of the sulcus and by other internal peculiarities, enables us to restore the missing parts of the sulcus with a high degree of probability, as indicated by the row of crosses in figure 3. But in the thalamus under the lobus medius thalami all criteria fail, the locus of the sulcus limitans being here obliterated internally, as well as externally (fig. 9).

The paired giant cells of Müller have been so fully described and pictured by various authors that nothing is necessary here beyond indicating on the diagram (fig. 3) their size and position. In addition to the five pairs of huge multipolar cells, there are in the motor column a considerable number of pale round cells much smaller than the giant cells, but still conspicuously larger than the ordinary cells of the motor column. They are commonly situated in the lateral border of the cell column and are usually unsymmetrically arranged.

Nervus oculomotorius. Three nuclei of the III nerve have been described in cyclostomes. Tretjakoff ('09, p. 678) in *Ammocoetes* mentions dorsal, ventral and lateral nuclei. The lateral nucleus

in our preparations consists of large pale multipolar cells scattered between the ventricular grey and the outer surface of the brain immediately rostrally of the emerging root fibers. These neurones closely resemble in form and position those of the nucleus ruber described by de Lange ('12) in a number of lower vertebrates; but Johnston ('02, p. 12) has traced their neurites directly into the III nerve. The dorsal and ventral nuclei of Tretjakoff are not clearly distinguishable in our preparations. Their cells are smaller than those of the lateral nucleus and are closely packed around the fourth pair of giant cells of Müller. The root fibers from these cells decussate in large numbers and form a ridge in the floor of the ventricle which forms the upper boundary of a small medial recess, the recessus oculomotorius (fig. 3, *r.III.*). The decussation of the III root fibers is separated by the entire width of this recess from the underlying commissura ansulata.

Sulcus medius. Mention has already been made (p. 642) of a sharp deep sulcus medius which runs longitudinally above the posterior end of the optic chiasma. From this point a wide shallow groove runs forward under the lobus subhippocampalis to the interventricular foramen. This groove resembles superficially the sulcus Monroi of Reichert in the human brain and was regarded by Herrick ('10, p. 470) as comparable with the sulcus diencephalicus medius of amphibian brains. From the fact that both the lobus subhippocampalis and the primordium of the corpus striatum between which this sulcus lies are in *Amphibia* evaginated into the cerebral hemisphere, it follows of course that this sulcus cannot as a whole be compared with either Reichert's sulcus Monroi or Herrick's sulcus medius. These sulci of higher brains (which are probably incompletely homologous), however, connect with the sulcus limitans of His in much the same way as does the longitudinal sulcus here in question. For descriptive purposes the term 'sulcus medius' will be applied to the entire length of this sulcus of *Ichthyomyzon*, with the reservation, however, that its homology with the amphibian sulcus so named is unproved and that in any event its anterior end cannot be so regarded.

Sulcus hypothalamicus. Johnston ('12, p. 349) describes our sulcus medius as part of a crescentic sulcus hypothalamicus extending from the interventricular foramen into the infundibulum. From the limited data now available it is by no means certain that the sulcus hypothalamicus which Johnston describes in cyclostomes is strictly comparable with his amphibian sulcus of the same name (sulcus diencephalicus ventralis of Herrick). In our model of *Ichthyomyzon* Johnston's sulcus hypothalamicus is completely interrupted between its horizontal and vertical limbs by the lobus ventralis thalami, and on the right side the vertical limb is represented by two distinct sulci, of which the more posterior corresponds with part of Johnston's sulcus hypothalamicus. We have designated these vertical grooves as sulcus hypothalamicus 1 and 2 respectively. On the left side of our model the relations are more like Johnston's model, the sulcus hypothalamicus 1 being fused below with sulcus hypothalamicus 2. How significant these individual variations may be it is impossible to determine from the limited material at hand.

Sulcus ventralis. This is a short longitudinal sulcus extending between the tuberculum posterius and the chiasma ridge, which was mentioned by Herrick ('10, p. 470). Johnston ('12) failed to distinguish it and considered it a part of his sulcus hypothalamicus; but our model shows it clearly on both sides as a well defined groove which crosses the hypothalamic sulci at approximately a right angle.

The optocoele is a deep lateral depression of the ventricular surface formed by the lateral evagination of the entire wall of the tectum mesencephali, its middle part reaching ventralward as far as the sulcus limitans. Its posterior wall is formed by two eminences, above by a thick tectal swelling which contains the posterior tectal commissure and below by a posterior tegmental swelling which crosses the site of the sulcus limitans and in this region obliterates this sulcus on the ventricular surface. The optocoele is bounded in front by the massive posterior commissure and a postcommissural ridge which is visible on both the ventricular and the lateral surfaces of the brain. Beneath the anterior part of this eminence and the recessus metathalam-

icus and above the sulcus limitans is a tegmental swelling which is directly continued forward into the lobus medius thalami.

Figure 11 illustrates the appearance of this region in cross section at the level of the rostral end of the posterior commissure and the caudal end of the mammillary recess. The sulcus limitans is clearly marked both by a ventricular groove and by a thickening of the ependyma. The cell plate which forms the ventricular grey is more irregularly arranged below the sulcus limitans and its neurones are larger and more angular. Above this sulcus is the tegmental swelling referred to, which appears to be formed by a thickening of the cell plate. Still farther dorsally the section passes through the caudal end of the metathalamic recess, which is bounded by a still thicker cell plate. In fact, this recess seems to have been formed by a slight total fold of the brain wall occasioned by rapid growth of the dorsal part of the cell plate. Above the recess is the postcommissural eminence and laterally of it the caudal end of the lateral geniculate body which contains the optic tract laterally and internally a dense neuropil containing numerous neurones which have apparently been derived by migration from the underlying thickened cell plate in the floor of the metathalamic recess.

The recessus pinealis is a narrow lateral pit in front of the posterior commissure whose roof and side walls are membranous.

The recessus metathalamicus is a wide saucer-shaped depression whose deepest part is immediately below the pineal recess. It is more extensive on the left side than on the right. Attention has already been called to the fact that this recess, like the optocoele, is apparently caused by a total outward fold of the brain wall, this part of the wall being roughly comparable with the metathalamus of higher brains. The grey matter in the wall of the anterior part of this recess, together with a part of the lobus subhabenularis adjacent, constitutes the nucleus secundus thalami of Schilling ('07, p. 433).

The designation of this region as metathalamic recess is based chiefly on its topographic relations. It is crossed laterally by the optic tracts, among which are a few scattered neurones, which probably represent a primordium of the corpus geniculatum

laterale (figs. 10 and 11; cf. Johnston '02, p. 29). Moreover, Tretjakoff describes ('09, p. 729) neurones of this region whose neurites pass into the postoptic decussation to end in the opposite thalamus, these fibers being comparable with the mammalian commissure of Gudden from the corpus geniculatum mediale.

The habenula has been briefly commented upon in connection with the description of the lateral surface.

Sulcus subhabenularis. On the left side the habenula is bounded below by a sharp deep crescentic subhabenular sulcus on the ventricular surface; but on the right side this condition prevails only under the anterior and posterior ends of the habenula. The middle part of the sulcus is partially obliterated by irregular swellings occupied by fibers of the large fasciculus retroflexus (figs. 4, 10) and by clusters of small cells which resemble those of the habenula.

Below the habenula there is a distinct eminence which has already been mentioned as visible on the lateral surface, the *lobus subhabenularis thalami*, which is present on both sides, though smaller on the left. On the left side it is smooth, but on the right it bears on the ventricular surface two vertical ridges. This lobe is traversed by the fasciculus retroflexus, which is small on the left side and exerts no influence on the ventricular surface. On the right side, though the fasciculus is very large and lies immediately adjacent to the ventricular ependyma which covers the subhabenular lobe (fig. 9), it does not produce either of the vertical ridges just mentioned. In fact, the thickest part of the fasciculus lies in the floor of the groove between the two ridges (sulcus thalamicus 3, see below). The development of the subhabenular lobe is undoubtedly due primarily to a proliferation of cells in this region (fig. 10), and the appearances strongly suggest that on the right side the enlargement of the fasciculus retroflexus results in a displacement forward and backward of some of these cells, thus producing the two vertical ridges. In front of the first of these ridges and separated from it by a light sulcus (sulcus thalamicus 2, cf. fig. 3) is another vertical ridge, the *eminentia thalami*, which is present in identical relations on both sides.

The subhabenular lobe is bounded below by a distinct longitudinal sulcus, here termed the *sulcus intermedius thalami* (fig. 3, *s.i.*), which communicates in front with the sulcus thalamicus 1 and behind with the recessus metathalamicus. The sulci which separate the three vertical ridges last described are named respectively the *first, second and third thalamic sulci* (fig. 3). The sulcus thalamicus 1 communicates below with the sulcus medius and above it forms the posterior boundary of the primordium hippocampi, terminating dorsally in the prehabenular recess, which in turn communicates anteriorly with the saccus dorsalis.

The *lobus medius thalami* occupies the mid-thalamic region (fig. 3, *l.m.*). It is bounded above by the sulcus intermedius and in front by the eminentia thalami and sulcus thalamicus 2. Behind, its upper part is bounded by the recessus metathalamicus and its lower part is continuous with the tegmentum above the sulcus limitans of His. Ventrally the sulcus medius forms part of the boundary, but the posterior part of the lobus medius is continuous with the dorsal part of the lobus ventralis. The nucleus primus thalami of Schilling ('07, p. 432) is composed of the cells of this lobe, and probably also those of the eminentia thalami. These cells are smaller and more diffusely arranged than are those of the lobus ventralis (figs. 8, 9).

The *lobus ventralis thalami* has already been mentioned as an eminence which crosses the site of the sulcus limitans of His, which is here obliterated (fig. 9).

The *eminentia thalami* is a narrow vertical ridge lying above the sulcus medius and in front of the lobus medius and lobus subhabenularis (figs. 3, 4, 7, *em.th.*). It is produced by a proliferation of nerve cells among which pass the fibers of the tractus olfacto-habenularis of the stria medullaris system. It forms part of a vertical ridge extending dorsalward from the sulcus medius (fig. 4) and terminating above in the eminentia fimbriae. The characteristic laminated arrangement of its cells is limited to the region between the sulcus ventralis and the sulcus intermedius. Above the latter sulcus is a lower eminence containing few cells and full of fibers of the stria medullaris system (fig. 7) and

still farther dorsally is the eminentia fimbriae. The latter resembles the habenula in structure and is traversed laterally by fibers from the primordium hippocampi to the habenula and medially by fibers of the tractus olfacto-habenularis from the nucleus preopticus and nucleus olfactorius medialis (p. 660.)

The commissural ridge which contains the optic chiasma and the postoptic commissure complex (see beyond) is enormously developed in *Ichthyomyzon*, and is here termed the 'chiasma ridge.' Between the chiasma ridge and preoptic recess below and the interventricular foramen above is a flat triangular preoptic area of the ventricular wall which contains the preoptic nucleus, the medial olfactory nucleus and the primordium of the corpus striatum. The apex of this triangle lies above the posterior end of the chiasma ridge and its base is formed by the lamina terminalis and anterior commissure.

The cross sections show a compact thick layer of small cells which crosses the medial plane bordering both the upper and the lower surface of the chiasma (figs. 5, 6). Both of these cell plates probably correspond with the *preoptic nucleus* of fishes and amphibians, the massive nucleus having been separated into two parts by the backward growth of the commissural ridge containing the optic chiasma and the postoptic commissure. The ventral plate which will here be termed the postoptic nucleus, is continuous below with the central gray of the hypothalamus whose cells are more diffusely arranged (figs. 6, 7).

The cell plate lying dorsally of the chiasma ridge, in the triangular area above referred to, likewise consists of a thick densely crowded collection of cells adjacent to the chiasma (the preoptic nucleus) and a more complex dorsal portion. The latter is separately differentiated only in the rostral three fourths of the triangular preoptic area.

In the dorsal part of this area, forming the ventral wall of the sulcus medius and the interventricular foramen, is the primordium of the corpus striatum. This is a well defined plate of rather large loosely arranged cells which form the floor of the foramen for a considerable distance laterally in the hemisphere (figs. 3, 4, 5, 6, *c.s.*).

At the level of figure 6 there is a narrow zone of small deeply staining cells between the preoptic nucleus and the corpus striatum which increases rapidly in width as it is followed forward. This is the *nucleus olfactorius medialis* (figs. 3, 5, *nuc. ol. m.*), which in front of the foramen becomes continuous with the great lobus olfactorius which composes the posterior part of the cerebral hemisphere.

The floor of the interventricular foramen is formed chiefly by the striatum, but in part anteriorly by the nucleus olfactorius medialis. Its rostral boundary is formed by the lamina terminalis and by a lobule which is said by Johnston to contain bulbar formation (doubtless correctly; our preparations do not permit a clear analysis of this structure). The roof of the foramen is formed chiefly by the fibers of the dorsal commissure, and its posterior wall by the lobus subhippocampalis, which is described below.

The hypothalamus. The exact dorsal boundary of the hypothalamus of cyclostomes is difficult to define on the basis of our present knowledge. This part of the brain evidently includes the greater part of the region below the chiasma ridge and the tuberculum posterius, its dorsal limit lying probably at about the level of the sulcus ventralis. It includes the walls of the postoptic recess, infundibular sac and corpus mammillare, and a massive lateral area which probably corresponds in part with the inferior lobes of higher fishes.

The primordium hippocampi. The structure and homologies of this part of the cyclostome brain have been very clearly established by Johnston in his recent paper ('12). He has failed, however, in our opinion, to determine precisely its ventral boundary and this has led to some errors of interpretation.

The primordium hippocampi forms the dorsal part of the massive brain wall between the epithalamus and the dorsal commissure in the lamina supraneuroporica. Its dorsal border is marked by a ridge containing nerve fibers of diverse sorts. Some of these connect in front with the dorsal commissure and some behind with the habenula. The membranous roof is attached to this ridge and if, as Johnston maintains, the whole of the pri-

mordium hippocampi is telencephalic, then the line of attachment of the membranous roof must be regarded as taenia fornicis (fig. 6, *t.f.*) in spite of its peculiar form relations. We have termed the fiber tract which borders this taenia the fimbria (figs. 3, 6, *fm.*), though it is very incompletely homologous with the mammalian fimbria. Like the latter, it carries fibers from the secondary olfactory area to the hippocampus, and also fibers from the hippocampus to the habenula (tractus cortico-habenularis medialis). But here, as in urodele Amphibia, this last connection is made much more directly than in mammals.

The arrangement of the neurones of the primordium hippocampi is very characteristic, as Johnston has shown; the determination of the limits of this structure is therefore easily accomplished. Our preparations show the characteristic lamina of hippocampal cells extending forward from the prehabenular recess nearly to the dorsal commissure. The ventral limit of this lamina is well defined and under the rostral half of the primordium this limit is marked by a sharp sulcus, the *sulcus subhippocampalis* (figs. 3, 6, *s.shp.*). In the caudal part of the primordium its characteristic cells extend farther ventralward and the limiting sulcus is not developed. The position of this ventral border is indicated in figure 3 by a row of crosses. The posterior border of the primordium hippocampi is marked by a deep groove, the *sulcus thalamicus* 1.

The lobus subhippocampalis. This is a well defined eminence lying below the primordium hippocampi (figs 3, 6, *l.shp.*). It is bounded above by the *sulcus subhippocampalis* (or by the corresponding *zona limitans* where this sulcus fails), below by the *sulcus medius*, in front by the *interventricular foramen*, and behind by the *sulcus thalamicus* 1. Its internal structure is very different from that of any of the surrounding parts, so that its limits could be easily defined even if there were no distinct ventricular sulci to mark them.

Behind the subhippocampal lobe is the *eminentia thalami*, whose cells are arranged in several parallel plates bordering the ventricular surface, with scattered cells farther peripherally (fig. 7). Passing forward from this region, as soon as the *sulcus*

thalamicus 1 is crossed and the subhippocampal lobe is entered, the cellular structure changes to a more diffusely scattered formation throughout the whole of this lobe. Dorsalward this diffuse formation is replaced by the hippocampal formation at the site of the sulcus subhippocampalis, and ventralward it is replaced by the corpus striatum at the sulcus medius (fig. 6). Lateralward the subhippocampal formation becomes continuous with the secondary olfactory area of the posterior lobe of the evaginated cerebral hemisphere.

From the preceding description it appears that the primordium hippocampi in *Ichthyomyzon* at no point comes into contact with the sulcus medius nor the underlying primordium of the striatum, but is separated from these structures for its entire length by the subhippocampal lobe. This relation is much more evident here than in the 120 mm. specimen previously studied. In this species the primordium hippocampi borders the inter-ventricular foramen to a very slight extent only. The posterior wall of the foramen is formed by the subhippocampal lobe, and the dorsal wall chiefly by the dorsal commissure. The cellular hippocampal formation extends forward into the dorsal commissure ridge only very slightly. The primordium hippocampi here does not bend "through the roof of the foramen to become directly continuous with the roof of the hemisphere," as described by Johnston ('12, p. 354). Unlike the primordial corpus striatum, which does bend through the floor of the foramen into the ventral wall of the evaginated hemisphere, the hippocampal formation is strictly limited to the unevaginated portion of the neural tube. The subhippocampal lobe, on the other hand, is directly continuous with the lobus olfactorius of the evaginated hemisphere.

The cerebral commissures. The internal structure of this brain has not been exhaustively studied, and only a brief reference will be made to the commissures and decussations shown by the model. The ventral cerebral commissural systems extend forward from the floor of the medulla oblongata to the lamina terminalis in an unbroken series except at three places, namely, the infundibulum, the postoptic recess and the preoptic recess.

In the floor of the mid-brain the very complex *ansulate commissure* system occupies the thick massive floor plate as far forward as the tuberculum posterius.

The entire massive wall of the recessus mammillaris contains commissural fibers. Anteriorly this commissural mass is somewhat thickened by fibers which appear to connect with the massive lateral walls of the infundibulum. This collection of fibers is termed the *post-infundibular commissure* (figs. 3, 9, 10, 11, *com.pi.*).

Between the infundibulum and the postoptic recess is a fold of the ventral wall of the brain which contains another mass of commissural fibers, the *preinfundibular commissure* (figs. 3, 5, *com.pri.*).

The optic chiasma and the postoptic commissures. The large commissural mass which is commonly termed the optic chiasma contains the decussation of the optic tracts and a much larger collection of commissural and decussating fibers which in the aggregate are termed the postoptic commissure, together with many nerve cells. The latter elements form the plates of cells in the central gray already referred to as crossing the medial plane on the dorsal and ventral borders of the chiasma ridge (fig. 5).

The fibrous masses lie between the two cell plates and of these masses the optic fibers form a relatively small proportion. The optic fibers are coarser than the others and so can readily be followed. Figure 5 illustrates their relations at their decussation, where they lie dorsally of the finer fibered postoptic commissure, and figure 3 indicates by a heavy line the full extent of the optic fibers in the medial plane. A cross section through the middle of the chiasma ridge is shown in figure 6, where it can be seen that the cell plates are considerably thicker than farther forward. All of the fibers here present in the medial plane belong to the postoptic commissure system, the optic tract lying farther dorsally at the lateral surface of the brain. This condition prevails throughout the remainder of the chiasma ridge until its extreme posterior end is approached, where the dorsal

and ventral cell-plate of the chiasma fuse with the post-chiasmatic vertical cell-plate of the lobus ventralis thalami (fig. 7).

In the later developmental stages the postoptic commissural system appears to increase in extent as compared with the larval condition, and the chiasma ridge to push much farther backward through the brain substance. In our model its posterior end reaches farther caudad than the transverse plane of the posterior end of the primordium hippocampi, while in Johnston's model of the 120 mm. specimen its posterior end lies notably farther forward with reference to both the primordium hippocampi and the hypothalamic structures.

The commissures hitherto mentioned lie in the floor-plate of the brain, that is, ventrally of the sulcus limitans of His, while the remaining commissures lie in the roof-plate above the sulcus.

The anterior commissure is a very slender strand of fibers in the lamina terminalis immediately in front of the nucleus olfactorius medialis (fig. 3, *com.a.*)

The dorsal olfactory commissure (commissura pallii anterior of Johnston '12; commissura olfactoria superior of Sterzi, '07) is a more massive fiber bundle crossing the lamina supra-neuroporica immediately in front of the primordium hippocampi (fig. 3, *com.d.*).

The superior commissure, or commissura habenularum, connects the posterior parts of the two habenular bodies (fig. 10, *com.s.*).

The posterior commissure is very massive, occupying the usual position immediately behind the pineal recess (figs. 3, 11, *com.post.*). The commissura tecti, which in higher brains occupies the remainder of the roof of the brain is here interrupted by the choroid plexus of the mid-brain roof and its fibers are in part concentrated in the posterior commissure and in larger numbers at the caudal end of the roof in a massive post-tectal bundle termed the dorsal decussation by Johnston ('02). The post-tectal mesencephalic decussation (fig. 3, *com.p.t.*) is continuous behind with the cerebellar commissure, the boundary between these being marked by the decussation of the fourth cerebral nerve.

DISCUSSION

The observations here reported were directed primarily toward the elucidation of certain facts regarding the external form and ventricular sculpturing of the brain of *Ichthyomyzon* in relation to the underlying deep structures, in the hope that these data may ultimately be of service in the interpretation of the morphogenetic factors which have operated in the evolution of the definitive form of the vertebrate brain. The brief reference to the brain of this species made by Herrick ('10) and the more thorough examination made by Johnston ('12) brought out some differences both of observation and interpretation which require control, and to these points attention will next be directed.

In the first place, as to terminology, we have here endeavored to select names for all parts, whose morphological significance is not quite definitely established, which are as objective and free from interpretative implication as possible. These topographic designations, such as *lobus medius thalami*, *lobus subhippocampalis*, etc., are of course purely provisional descriptive terms to be abandoned whenever definite homologies with higher brains can be determined. It is not improbable that for some of these parts such homologies can never be established; for some of the structures here enumerated are doubtless expressions of cenogenetic functional differentiation, rather than vestiges of primary segmental or other primitive morphological relations, and identity of structural pattern in such functional adaptations is not to be expected when aberrant members of the phylogenetic series are directly compared.

A fundamental problem in such studies is, accordingly, to differentiate between such structures as the *sulcus limitans* of His, which mark very ancient and primitive relations, and functional adaptations of more recent origin and limited occurrence. Obviously the first step is the determination of the exact form and functional connections of all the parts in question in a large series of vertebrate types and developmental stages before such generalizations can safely be attempted. And our knowledge of the internal structure of the diencephalon of cyclostomes (and

this applies to all of the true fishes also) is as yet too meager to warrant any but provisional morphological interpretations.

Recent investigations, particularly those of Sterzi and Johnston, have established with a high degree of probability the homologies of the subdivisions of the telencephalon of cyclostomes. The evaginated cerebral hemisphere includes an anterior lobe, the olfactory bulb, and a posterior lobe containing a portion of the secondary olfactory area and a portion of the primordium of the corpus striatum. The secondary olfactory area (nucleus olfactorius) of mammals has three divisions, lateral, medial and intermediate. The intermediate nucleus, or tuberculum olfactorium (lobus parolfactorius of Edinger), has not been identified in cyclostomes. The medial nucleus is represented in the ventro-medial part of the cyclostome hemisphere and in a portion of the unevaginated telencephalon medium between this region and the preoptic recess. The lateral olfactory nucleus is represented in the posterior lobe of the hemisphere, though it cannot be maintained that these two structures are strictly homologous.

The primordial striatum lies in the floor of the interventricular foramen partly within the telencephalon medium and partly within the hemisphere, exactly as in young amphibian larvae. The remainder of the telencephalon lies entirely in the unevaginated portion of the neural tube and includes the preoptic nucleus, the primordium hippocampi and the lobus subhippocampalis. The first of these structures remains throughout the vertebrate series in the telencephalon medium; the other two are variously arranged in different species of fishes, and in amniote vertebrates are fully evaginated in the cerebral hemisphere.

The structure here termed 'lobus subhippocampalis' was first described by Herrick ('10, p. 472) as the rostral end of the pars dorsalis thalami, the overlying primordium hippocampi there being termed the dorso-median ridge. In the 120 mm. specimen of *Ichthyomyzon* there described no sulcus was found separating these two regions, but they were distinguished on the basis of internal structure. Johnston ('12) in reexamining the same material did not observe this internal differentiation and described both regions as primordium hippocampi, whose ventral boundary

was therefore placed at our sulcus medius (his sulcus limitans hippocampi). The larger specimen which forms the basis of the present report, however, shows not only a much clearer internal difference between these regions but throughout most of their extent a well defined ventricular sulcus between them, the sulcus subhippocampalis. Our sulcus medius, therefore, is not the limiting sulcus of the hippocampus, nor is the latter (the sulcus subhippocampalis) to be compared with Elliot Smith's sulcus limitans hippocampi of mammals, for the latter separates the hippocampus from the medial olfactory area in the septum of an evaginated hemisphere, structures which here are separated by both the lobus subhippocampalis and the striatum (cf. Herrick '10, p. 465).

The evidence recently brought forward by Johnston ('12) renders it probable that in Herrick's discussion of the cyclostome brain ('10, p. 473) the di-telencephalic boundary was placed too far forward. It probably lies in our specimen in about the plane of the sulcus thalamicus 1, thus defining both the primordium hippocampi and the lobus subhippocampalis as telencephalic structures.

The homologies of the lobus subhippocampalis in higher brains can be definitely determined only after its functional connections in cyclostomes are better known and the method by which the process of evagination of the cerebral hemispheres was accomplished has been definitely determined. Even in the case of the primordium hippocampi, comparisons with the hippocampus of higher brains should be made with caution. That this is the region from which the amphibian and the amniote hippocampus has been differentiated seems well established; but the relations of this structure and the lobus subhippocampalis to each other and to the mammalian hippocampus and pyriform lobe (among other questions) require further elucidation.

The morphological problems presented by the diencephalon of cyclostomes are more obscure than those of the telencephalon, chiefly by reason of our very imperfect knowledge of the structure of the thalamus in all lower vertebrates. That there is a considerable regional functional differentiation within the thalamus of

cyclostomes has been emphasized by Schilling ('07, p. 432) and is evident from our preparations, but our material is inadequate for an analysis of these relations.

The most important landmark here is undoubtedly the sulcus limitans of His, whose diencephalic portion is not, however, clearly preserved in our specimen. As already stated (p. 642), this sulcus appears to be coextensive with the posterior part of our sulcus medius and farther anteriorly with a shallow depression which follows the upper border of the chiasma ridge into the preoptic recess. In the mid-thalamic region this sulcus is entirely interrupted by an eminence which is here termed the lobus ventralis thalami. The relations of this lobe are substantially identical so far as known with those of the pars ventralis thalami of amphibian larvae (Herrick '10) and we incline to regard these structures as homologous. In both cases this eminence is formed by the multiplication and differentiation of subependymal neurones in a region which crosses the site of the primary sulcus limitans, which is thereby obliterated. Here it clearly lies chiefly below the site of the sulcus, which was doubtless its primitive position; that is, it was first differentiated in the ventro-lateral or motor lamina of the neural tube. But even in this cyclostome it has extended sufficiently far dorsalward to obliterate the sulcus in this region—a process of differentiation which appears to have advanced much farther in the Amphibia. This structure lies for the most part behind the region designated *pars ven. thal.* in our earlier account (Herrick '10, fig. 73) the latter referring chiefly to the posterior part of the corpus striatum and to an intervening undifferentiated area. The comparison of any part of this structure with the eminentia thalami of urodeles (cf. Herrick '10, p. 471) is obviously untenable, as Johnston has pointed out ('12, p. 349).

Johnston describes ('12, p. 347) in Ichthyomyzon, as in other cyclostomes, a crescentic sulcus hypothalamicus extending from the interventricular foramen backward and downward into the hypothalamus. There is no such continuous sulcus on either side of our specimen, the horizontal limb and vertical limb of Johnston's sulcus being entirely interrupted by the lobus ven-

tralis thalami. The horizontal limb of this sulcus is our sulcus medius. Johnston states ('12, p. 349) that this is a part of his sulcus limitans hippocampi and that Herrick's sulcus ventralis is the ventral part of his sulcus hypothalamicus. Neither of these statements is supported by our present examination. We have already seen that the sulcus medius and the limiting sulcus of the hippocampus (sulcus subhippocampalis) are separated by the subhippocampal lobe; and our sulcus ventralis is a longitudinal groove which cuts across the hypothalamic sulci at almost a right angle.

The opinion was expressed by the senior author (Herrick '10, p. 471) that the sulcus medius and the sulcus ventralis are comparable with the sulci so named by him in the Amphibia. The solution of this question must be reserved until fuller knowledge of the functional connections of the related parts of the cyclostome brain is obtained, and the terms are here used in a descriptive sense merely.

In higher brains the thalamus proper is clearly divided into a dorsal part which receives the lemniscus, optic tracts, etc., and is in the main a cortical dependency, and a ventral part more directly concerned with motor or emissive functions. As far down the animal scale as the Amphibia this distinction is very evident, though in the latter case the sensory correlation centers of the dorsal part of the thalamus have small cortical connections. How far this type of differentiation prevails in the thalamus of fishes must await further study. To us it appears not improbable that even in cyclostomes the medial and ventral lobes of the thalamus as here described have been differentiated in accordance with the same functional factors as are evident in the dorsal and ventral parts respectively of the amphibian thalamus.

The status of the subhabenular lobe is obscure. The meager data at hand suggest that it is a habenular dependency, but whether it belongs primarily in the thalamus or the epithalamus must be determined by further study. Tretjakoff ('09, p. 732) describes fibers of the stria medullaris system from the secondary olfactory area as ending in the thalamus (probably in our subhabenular lobe), partly uncrossed and partly after decussation

in the commissura superior. This suggests a very intimate relation between this region and the habenula.

Johnston ('12, p. 345) describes two vertical ridges under the habenula, which he says are related respectively to the stria medullaris and the tractus habenulo-peduncularis (fasciculus retroflexus of Meynert) and are separated by a vertical sulcus termed 'sulcus medius.' The latter he compares with the amphibian sulcus medius of Herrick. In *Ichthyomyzon* his sulcus medius (p. 349, and fig. 6) evidently corresponds with our sulcus thalamicus 3. His eminentia thalami is divided by a small sulcus which he regarded as an artefact (p. 377) into two vertical ridges. This sulcus is our sulcus thalamicus 2. The anterior part of Johnston's eminentia thalami corresponds to our eminence of the same name. Its posterior part and his ridge related to the tractus habenulo-peduncularis form our subhabenular lobe, these relations being confirmed by a study of the left side of our model, where the tracts related to the habenula are smaller and thus permit a better analysis of the cellular masses (cf. p. 647).

The eminentia thalami was first defined by Herrick ('10, p. 419) in the Amphibia. Its functional connections are still imperfectly understood, but dendrites of its neurones are known to be related to fibers of the adjacent stria medullaris and the neurites of these cells in Amphibia pass backward into the pars ventralis thalami. For these reasons it was regarded as functionally related to the pars ventralis thalami, from which, however, it is separated in the urodele amphibians by a well defined vertical sulcus, and from which it differs greatly in internal structure. The corresponding structure in *Ichthyomyzon* appears to be divided into two parts, a ventral and a dorsal, both of which are differently related to the other parts of the diencephalon than in Amphibia by reason of the peculiar relations of the stria medullaris in cyclostomes.

The components which make up the stria medullaris of higher brains appear to be separated in the petromyzonts into two imperfectly distinct groups, a ventral and a dorsal (Johnston '12, p. 358). The ventral group of fibers arise chiefly from the nucleus preopticus, nucleus olfactorius medialis and lobus olfactorius and

pass directly dorsalward, through and behind the primordium hippocampi. The neurones of the eminentia thalami of our description of *Ichthyomyzon* are differentiated chiefly in relation with this system of stria medullaris fibers. This structure is well seen in our figure 4 and in Johnston's figures of *Lampetra* ('12, fig. 5, *em.th.*).

The dorsal group of stria medullaris fibers passes through the substance of the primordium hippocampi, chiefly in the tract which we have termed the fimbria, and related with these fibers is the more dorsal eminence under the habenula which we term the eminentia fimbriae (figs. 3, 4, 7, *em.f.*). This structure is seen in Johnston's figures of *Ichthyomyzon* ('12, figs. 6, 9, *em.th.*). Whether these two eminences are of common physiological type has not been determined. If the primary functional connection of the eminentia thalami is with the stria medullaris, as seems probable, its form and topographic relations with other cerebral structures will necessarily vary with the course of those fibers. Accordingly, it cannot be used as a fixed landmark in morphological interpretations of other unrelated parts of the brain.

Our examination of the internal structure of this cyclostome brain has led us to conclude that the superficial form of the external and ventricular surfaces is the expression of focal differentiations which are functionally determined. Just how far these superficial landmarks as thus defined coincide with primitive morphological factors, such as metamerism, and so forth, can be determined only by further embryological and comparative studies. Probably the most fundamental landmark in the vertebrate brain is the sulcus limitans of His, and it has evidently been functionally determined.

In that portion of the cyclostome brain which lies in front of the isthmus the neurones tend to be arranged in a tolerably compact layer of cells (central gray matter) on each side of the ventricle. In different regions under the influence of diverse functional connections this layer breaks up into special laminae of cells, which are often structurally differentiated from their neighbors. The next step in this differentiation is the migration of neurones from these laminae of central gray to form more super-

ficial areas of diffusely arranged cells, an arrangement seen, for instance, in the lateral geniculate body of the recessus metathalamicus.

The different cell laminae vary in thickness and in the number of contained neurones. Where these thickenings are localized and not very extensive they produce ventricular eminences, the interverning sulci representing simply lines of less extensive proliferation of neurones and of relatively indifferent physiological type. Thus are formed the lobus medius and lobus ventralis thalami and some other ventricular eminences. In some of the larger areas of this sort the increase of the thickness of the brain wall may be so marked as to cause an externally visible eminence on the lateral surface of the brain also, as in the case of the lobus subhabenularis and primordium hippocampi. But if the enlargement of the cell plate is carried still further, there results a total fold of the brain wall, marked by an external eminence and a ventricular recess, as seen in the tectum mesencephali, and elsewhere.²

In any comparison of the ventricular sculpturing of a cyclostome brain with other species, these functional factors must first be determined and compared and the embryological development must also be investigated in order that the influence of vestigial factors may be recognized. We are very far from a sufficiently complete knowledge of the cerebral structure of any ichthyopsid types to justify final conclusions and our morphological interpretations of these structures should be regarded as provisional only until our anatomical knowledge is more complete.

CONCLUSIONS

This inquiry was directed primarily toward a detailed examination of the external and ventricular surfaces of a lowly organized type of brain and of the underlying deep structures, as an aid to the understanding of the functional factors which have operated in the evolution of brain form. In the cerebrum of petromyzonts (i.e., that portion of the brain in front of the isth-

²In this connection attention should be called to the recent illuminating analysis of the morphogenetic factors operative in the evolution of the mammalian cerebral cortex by Kappers ('13, pp. 368-372).

mus) the neurones are arranged for the most part very simply in a compact layer of central gray. This layer, however, is broken up into a series of detached cell plates, each with a characteristic type of form, size and arrangement of neurones, and this differentiation has in the main been functionally determined.

In the different parts of the adult brain of *Ichthyomyzon* various forms of functional differentiation of the primordial central gray can be observed and provisional conclusions can be drawn regarding the probable sequence of the ontogenetic and phylogenetic developmental stages of the corresponding parts of higher brains. These conclusions must be controlled by further study, especially of the details of embryonic development. The observations here recorded suggest that the first step in the functional differentiation is an increase in the number of neurones in the affected region, accompanied usually by adaptive changes in their form and internal structure.

A very slight thickening of the central gray may be manifested by a localized eminence on the ventricular surface which may be accompanied by an increase in the amount of neuropil in the overlying stratum album causing a similar eminence on the lateral surface of the brain. Further differentiation may be accompanied by the migration of neurones laterally from the central gray into this neuropil, thus giving rise to a more superficial nidulus (nucleus) among the terminals of the afferent tract related to the area in question, in accordance with the doctrine of neuro-biotaxis (Kappers). An early phase of this type of differentiation is seen in the lateral geniculate body of *Ichthyomyzon*.

Further increase in the number of neurones in the differentiated area results in an outward folding of the entire wall of the neural tube, thus producing a lateral evagination, as illustrated in the tectum mesencephali and cerebral hemisphere of *Ichthyomyzon*. Here too there may follow a lateral migration of neurones away from the central gray, producing a layer of superficial gray matter. Both of the latter processes attain their maximum in the mammalian cerebral hemisphere.

The details of the form changes involved in the process of evagination of the cerebral hemispheres of higher brains are as

yet undetermined, and the first step in the solution of this problem is a more exact determination of the corresponding structures in the unevaginated telencephalon medium of early phylogenetic and ontogenetic stages.

In Ichthyomyzon the evaginated hemisphere includes the entire primary olfactory area (olfactory bulb), the greater part of the secondary area and a smaller part of the corpus striatum. In the telencephalon medium there is left the primordium hippocampi, a subhippocampal lobe and a part of the corpus striatum, all of which are completely evaginated in higher brains, and also a portion of the secondary olfactory area. The posterior part of the latter is preserved in the telencephalon medium of the highest brains (probably here as an olfactory center of the third order) in the preoptic nucleus ('ganglion basale opticum' of authors).

The ventricular sculpturing of the diencephalon has been fully described and comparisons made with Johnston's recent account. It is suggested that the homologies of these structures in fishes cannot be definitely determined until fuller comparative data regarding their functional connections are available.

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REFERENCE LETTERS

- b.ol.*, bulbus olfactorius
cb., cerebellum
c.gen.lat., corpus geniculatum laterale
ch., chiasma optica
c.mam., corpus mammillare
com.a., commissura anterior
com.ans., commissura ansulata
com.d., commissura olfactoria dorsalis
com.pi., commissura postinfundibularis
com.po., commissura postoptica
com.post., commissura posterior
com.pri., commissura preinfundibularis
com.p.t., commissura posterior tecti
com.s., commissura superior
c.s., primordium of corpus striatum
em.f., eminentia fimbriae
em.pc., eminentia postcommissuralis
em.th., eminentia thalami
ep., epiphysis
ep.s., epiphyseal stalk
F., foramen interventriculare
fim., fimbria
f.retrof., fasciculus retroflexus Meynerti
gl.cl., glomerulus olfactorius
hab., habenula
hyp., hypophysis
hyth., hypothalamus
inf., infundibulum
isth., isthmus
l.m., lobus medius thalami
l.ol., lobus olfactorius
l.sh., lobus subhabenularis
l.shp., lobus subhippocampalis
l.t., lamina terminalis
l.v., lobus ventralis thalami
n.II, nervus opticus
n.III, nervus oculomotorius
n.IV, nervus trochlearis
nuc.ol.m., nucleus olfactorius medialis
nuc.po., nucleus preopticus
nuc.posto., nucleus postopticus
n.V., nervus trigemini
n.VII + VIII, nervi facialis et acusticus
optc., optocoele
ped., pedunculus cerebri
prim.hip., primordium hippocampi
r.III, recessus oculomotorius
r.m., recessus metathalamicus
r.mam., recessus mammillaris
r.o., recessus postopticus
r.pin., recessus pinealis
r.po., recessus preopticus
sac.d., saccus dorsalis
sac.v., saccus vasculosus
s.hy.1 and *s.hy.2*, sulcus hypothalamicus 1 and 2
s.i., sulcus intermedius
s.l., sulcus limitans
s.m., sulcus medius
s.sh., sulcus subhabenularis
s.shp., sulcus subhippocampalis
s.th.1, *s.th.2* and *s.th.3*, sulcus thalamicus 1, 2 and 3
str.med., stria medullaris
s.v., sulcus ventralis
tect., tectum mesencephali
tegm., tegmentum
t.f., taenia fornicis
t.m., taenia mesencephali
t.p., tuberculum posterius
tr.op., tractus opticus
t.th., taenia thalami
t.v.q., taenia ventriculi quarti
v.l., ventriculus lateralis
v.tr., velum transversum
1 to 5, the first five pairs of giant cells of Müller

Fig. 1 Dorsal view of a wax model of the cerebrum and rostral end of the rhombencephalon of *Ichthyomyzon concolor* (Kirtland). $\times 50$. The model was made at a magnification of 75 diameters and the illustrations were drawn by Mr. A. B. Streedain the same size as the model. Figures 1 to 4 were reduced to two-thirds of the dimensions of the drawings and figures 5 to 12 were reduced to one-half.

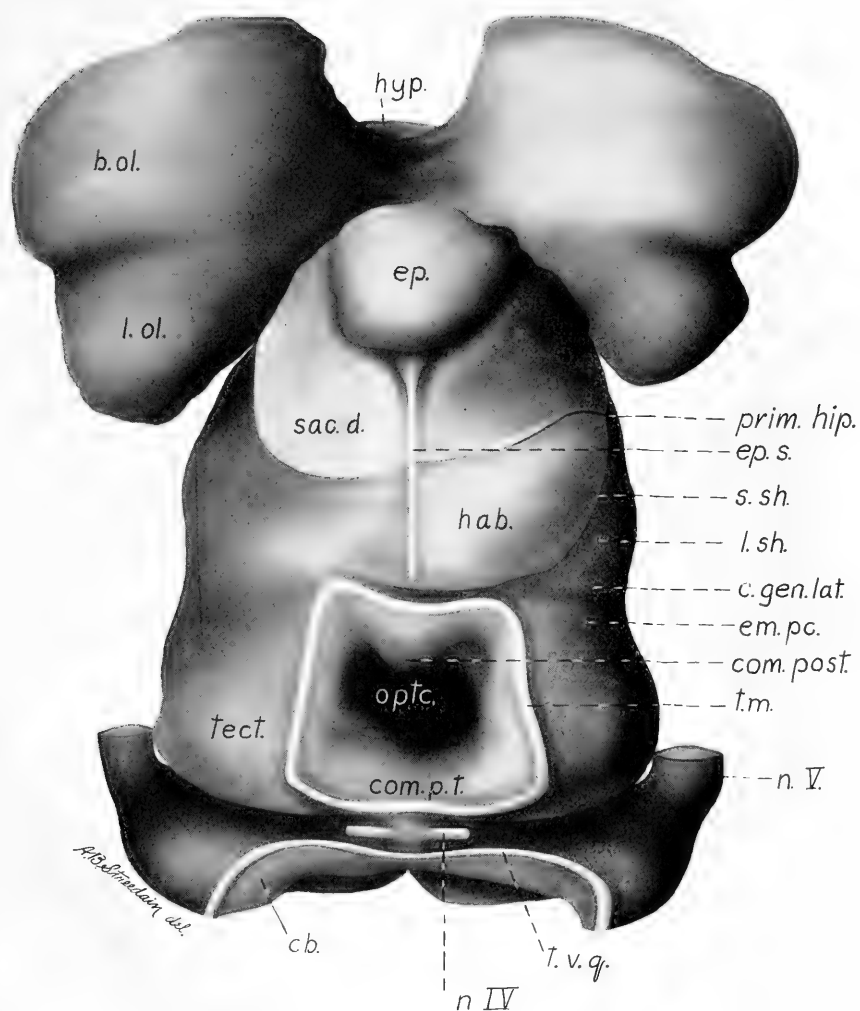
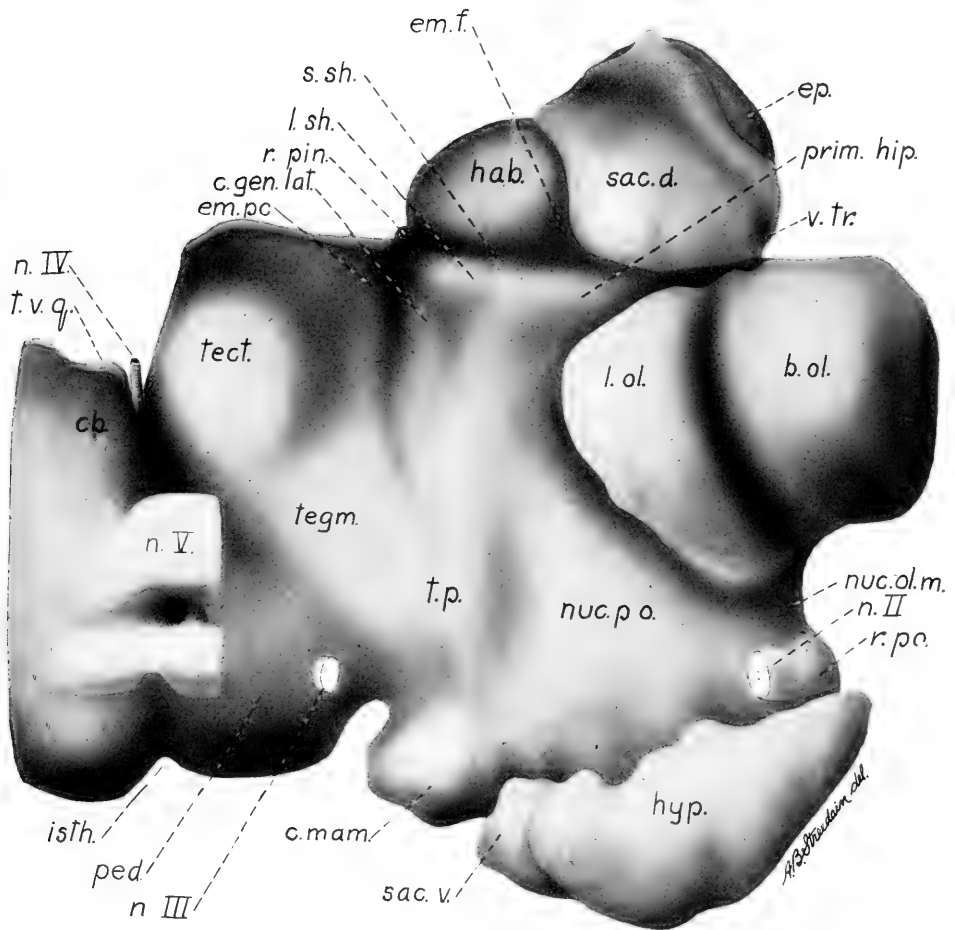


Fig. 2 Lateral view of the right side of the model. $\times 50$.



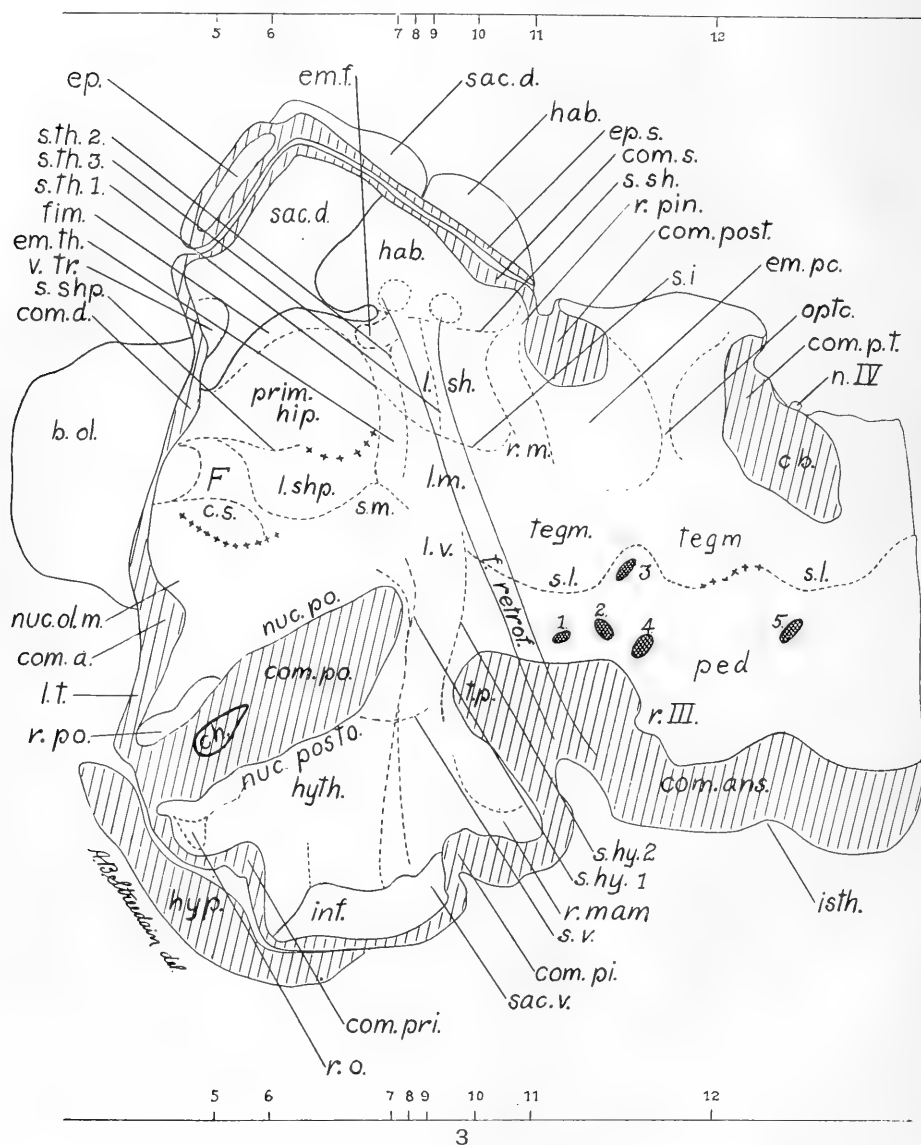
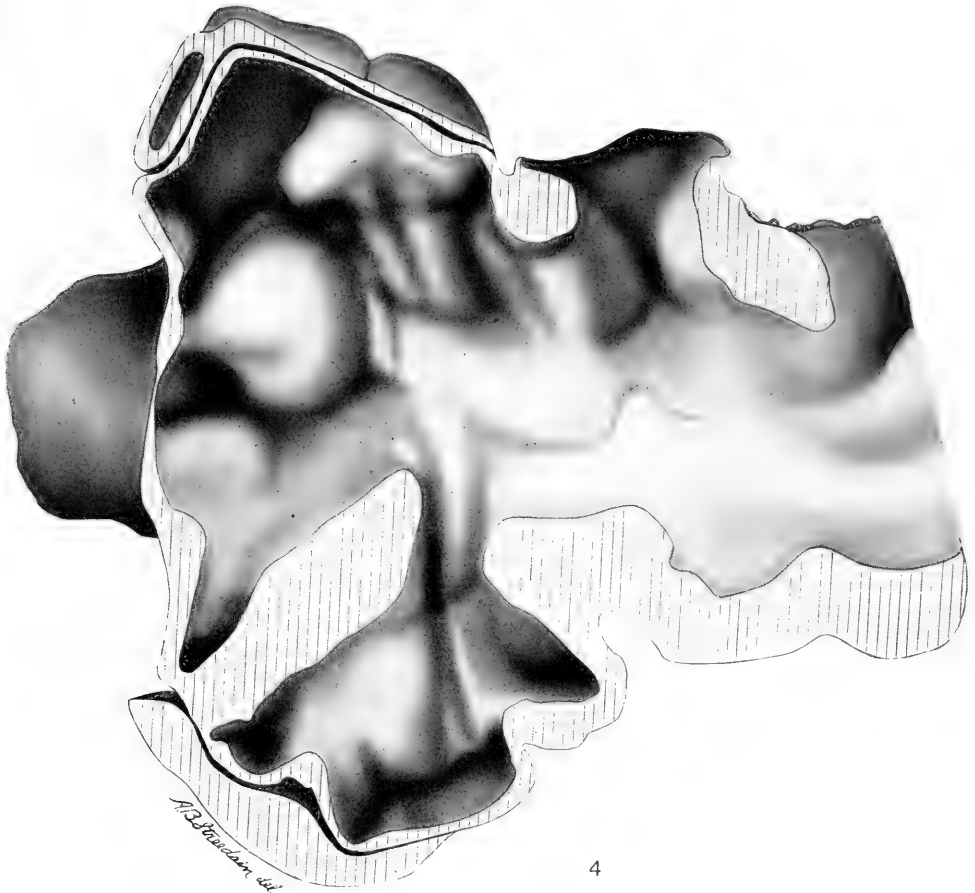


Fig. 3 Key drawing to accompany figure 4. The principal ventricular sulci are indicated by broken lines. Those portions of the sulcus limitans (*s.l.*) and the sulcus subhippocampalis (*s. shp.*) which are not evident on the ventricular surface but whose morphological positions can be definitely determined by the



underlying deep structures are indicated by rows of crosses. Similarly, the ventral boundary of that portion of the cell plate constituting the primordial corpus striatum (*c.s.*) which lies contiguous to the ependymal surface is indicated by a row of crosses. This cell plate extends farther ventrally and posteriorly than here indicated, but separated from the surface by other structures. The position of the fasciculus retroflexus of Meynert (*f. retrof.*), or tractus habenulopeduncularis, is also shown as projected upon the ventricular surface. The five giant cells of Müller which lie within the limits of the model are indicated by double cross-hatched areas (1 to 5). The epiphyseal stalk (*ep.s.*) is drawn somewhat exaggerated in size and farther removed from the membranous wall of the dorsal sac than is natural. On the scales at the top and bottom of the figure are indicated the levels at which figures 5 to 12 were taken.

Fig. 4 View of the medial surface of the right half of the model after division in the sagittal plane. $\times 50$.

Figs. 5 to 12 Comprise series of cross sections through the brain of *Ichthyomyzon* at the levels indicated on figure 3. $\times 36$.

Fig. 5 Section passing through the interventricular foramen and decussation of the optic tracts.

Fig. 6 Section passing through the primordium hippocampi and lobus subhippocampalis a short distance behind the interventricular foramen. The lamina of cells representing the primordial corpus striatum (*c.s.*) is extensively developed, though for the most part it is withdrawn from the ventricular surface.

Fig. 7 Section passing through the caudal end of the chiasma ridge. On the right side it passes through the habenula and eminentia fimbriae; on the left side through the caudal end of the primordium hippocampi. The cellular elements of the eminentia thalami (*em. th.*) are well developed between the sulcus medius and the sulcus intermedius. Between this region and the eminentia fimbriae is an area containing fibers of the stria medullaris and poor in cells, which should probably be associated with the lobus subhabenularis.

Fig. 8 Section through the middle of the right habenula and the rostral part of the thalamus.

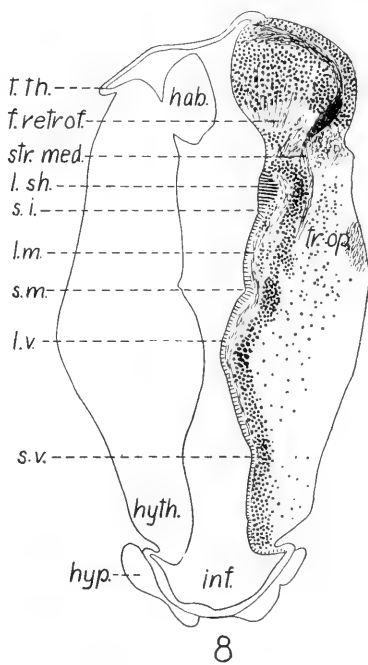
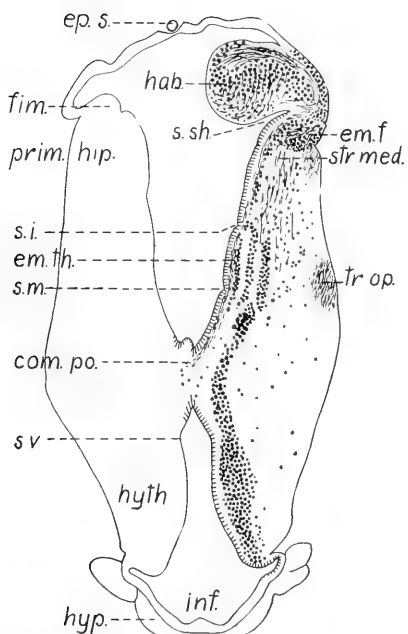
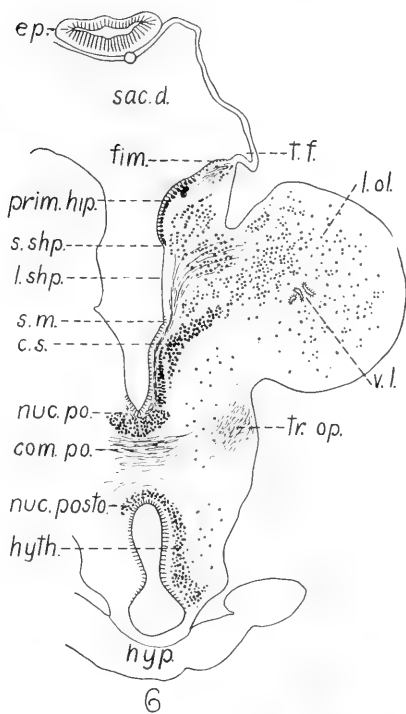
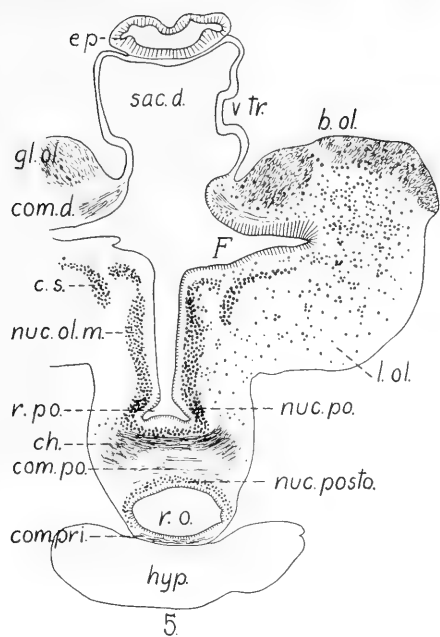
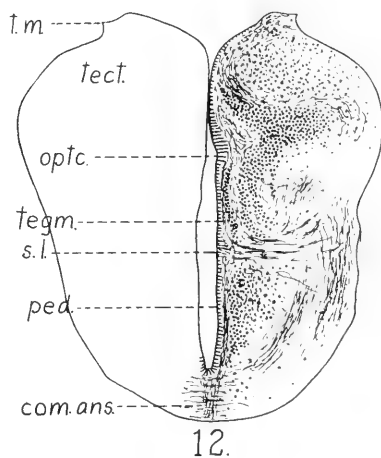
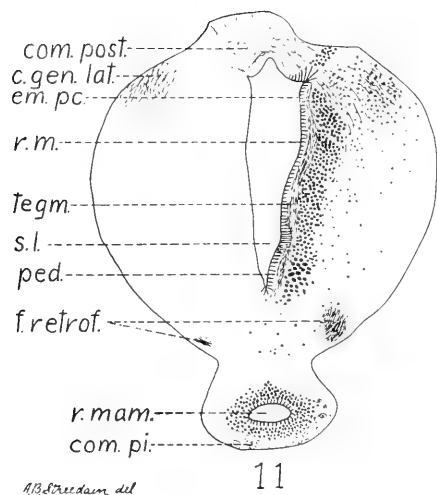
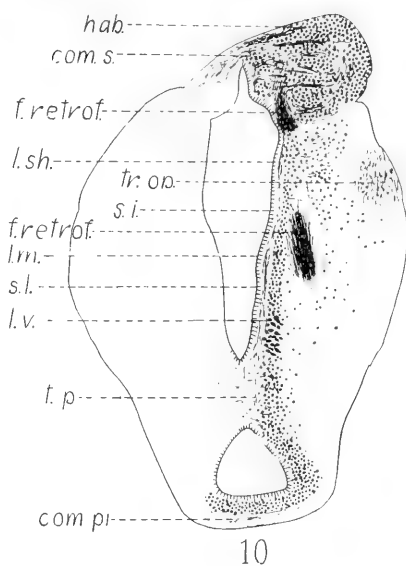
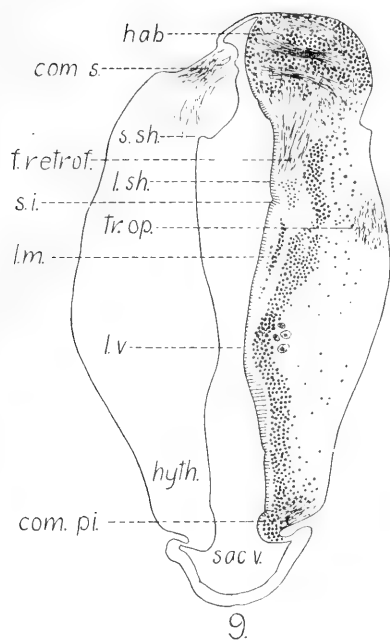


Fig. 9 Section through the thalamus three sections ($45\ \mu$) farther caudad than figure 8 and passing through the common vertical ridge formed by the medial and ventral lobes of the thalamus.

Fig. 10 Section through the rostral end of the tuberculum posterius. The letters *s.l.* mark the position of the sulcus limitans, which appears clearly defined a few sections farther caudad. The elements of the cell plate below this level are somewhat larger and more loosely arranged than those above it.

Fig. 11 Section through the posterior commissure and the caudal end of the recessus mammillaris.

Fig. 12 Section through the midbrain immediately in front of the commissura posterior tecti, illustrating the characteristic internal structure at the site of the sulcus limitans, through the sulcus itself is not evident on the ventricular surface (cf. fig. 3).



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